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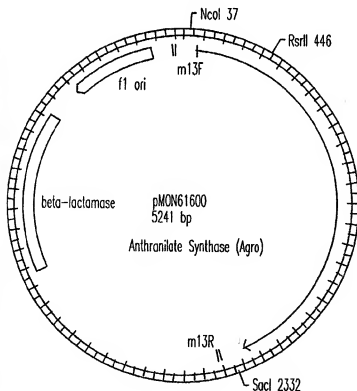
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(54) Title: TRANSGENIC HIGH TRYPTOPHAN PLANTS



(57) Abstract: The present invention provides a method for conferring tolerance to an amino acid analog of tryptophan to a plant and/or altering the tryptophan content of a plant by introducing and expressing an isolated DNA segment encoding an anthranilate synthase in the cells of the plant. Transgenic plants transformed with an isolated DNA segment encoding an anthranilate synthase, as well as human or animal food, seeds and progeny derived from these plants, are also provided.

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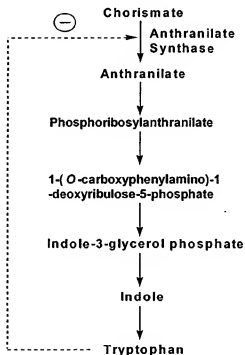
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TRANSGENIC HIGH TRYPTOPHAN PLANTS

Background of the Invention

The seeds of a number of important crops, including soybean and maize do not contain sufficient quantities of several amino acids to be nutritionally complete. These amino acids include, but are not limited to: tryptophan, isoleucine, valine, arginine, lysine, methionine and threonine. Therefore, the biosynthetic pathways for these amino acids, and/or biosynthetic pathways for metabolites that feed into those pathways, are potential targets for manipulation in order to increase the amino acid content of these plants.

Anthranilate synthase (AS, EC 4.1.3.27) catalyzes the first reaction branching from the aromatic amino acid pathway to the biosynthesis of tryptophan in plants, fungi, and bacteria.



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The most common form of anthranilate synthase (for example, the maize anthranilate synthase) is a heterotetrameric enzyme consisting of two subunits, the α or TrpE subunit and the β or TrpG subunit. Two α subunits and two β subunits assemble to form the heterotetrameric anthranilate synthases.

20 "Monomeric" forms of AS have also been discovered that comprise a single

polypeptide chain having the activities of both TrpE and TrpG subunits (for example *Rhizobium meliloti*). While monomeric anthranilate synthases comprise just one type of polypeptide, the enzymatically active form of a monomeric anthranilate synthase is typically a homodimer consisting of two such monomeric polypeptides. Both heterotetrameric and monomeric anthranilate synthases catalyze the formation of anthranilate in a reaction utilizing glutamine and chorismate. The domain found on the α subunit (referred to herein as the " α domain") binds chorismate and eliminates the enolpyruvate side chain, and the domain found on the β -subunit (referred to herein as the " β domain") transfers an amino group from glutamine to the position on the chorismate phenyl ring that resides between the carboxylate and the enolpyruvate moieties.

The next reaction in the synthesis of tryptophan is the transfer of the phosphoribosyl moiety of phosphoribosyl pyrophosphate to anthranilate. The indole ring is formed in two steps involving an isomerization converting the ribose group to a ribulose followed by a cyclization reaction to yield indole glycerol phosphate. The final reaction in the pathway is catalyzed by a single enzyme that may contain either one or two subunits. The reaction accomplishes the cleavage of indole glyceraldehyde-3-phosphate and condensation of the indole group with serine (Umbarger, *Ann. Rev. Biochem.* 47, 555 (1978)).

Metabolite flow in the tryptophan pathway in higher plants and microorganisms is apparently regulated through feedback inhibition of anthranilate synthase by tryptophan. Tryptophan may block the conformational rearrangement that is required to activate the β -domain and to create a channel for passage of ammonia toward the active site of the α -domain. Such feedback inhibition by tryptophan is believed to depress the production of tryptophan by anthranilate synthase. See Li J. & Last, R.L. The *Arabidopsis thaliana trp5* mutant has a feedback-resistant anthranilate synthase and elevated soluble tryptophan. *Plant Physiol.* 110, 51–59 (1996).

Several amino acid residues have been identified as being involved in the feedback regulation of the anthranilate synthase complex from *Salmonella typhimurium*. Such information provides evidence of an amino-terminal regulatory site. *J. Biol. Chem.* 266, 8328–8335 (1991). Niyogi et al. have

further characterized the anthranilate synthase from certain plants employing a molecular approach. See Niyogi and Fink (Plant Cell, 4, 721 (1992)) and Niyogi et al. (Plant Cell, 5, 1011 (1993)). They found that the α -subunits of the *Arabidopsis* anthranilate synthase are encoded by two closely related, nonallelic
5 genes that are differentially regulated. One of these α -subunit genes, ASA1, is induced by wounding and bacterial pathogen infiltration, implicating its involvement in a defense response, whereas the other α -subunit gene, ASA2, is expressed at constitutive basal levels. Both predicted proteins share regions of homology with bacterial and fungal anthranilate synthase proteins, and contain
10 conserved amino acid residues at positions that have been shown to be involved in tryptophan feedback inhibition in bacteria (Caligiuri et al., J. Biol. Chem., 266, 8328 (1991)).

Amino acid analogs of tryptophan and analogs of the intermediates in the tryptophan biosynthetic pathway (e.g., 5-methyltryptophan, 4-methyltryptophan,
15 5-fluorotryptophan, 5-hydroxytryptophan, 7-azatryptophan, 3 β -indoleacrylic acid, 3-methylantranilic acid), have been shown to inhibit the growth of both prokaryotic and eukaryotic organisms. Plant cell cultures can be selected for resistance to these amino acid analogs. For example, cultured tobacco, carrot, potato, corn and *Datura innoxia* cell lines have been selected that are resistant to
20 growth inhibition by 5-methyltryptophan (5-MT), an amino acid analog of tryptophan, due to expression of an altered anthranilate synthase.

Ranch et al. (Plant Physiol., 71, 136 (1983)) selected for 5-MT resistance in cell cultures of *Datura innoxia*, a dicot weed, and reported that the resistant cell cultures contained increased tryptophan levels (8 to 30 times higher than the
25 wild type level) and an anthranilate synthase with less sensitivity to tryptophan feedback inhibition. Regenerated plants were also resistant to 5-MT, contained an altered anthranilate synthase, and had greater concentrations of free tryptophan (4 to 44 times) in the leaves than did the leaves of the control plants. In contrast to the studies with *N. tabacum*, where the altered enzyme was not
30 expressed in plants regenerated from resistant cell lines, these results indicated that the amino acid overproduction phenotype could be selected at the cellular level and expressed in whole plants regenerated from the selected cells in *Datura innoxia*.

Hibberd et al. (U.S. Patent No. 4,581,847, issued April 15, 1986) described 5-MT resistant maize cell lines that contained an anthranilate synthase that was less sensitive to feedback inhibition than wild-type anthranilate synthase. One 5-MT resistant cell line accumulated free tryptophan at levels
5 almost twenty-fold greater than that of non-transformed cell lines.

P. C. Anderson et al. (U.S. Pat. No. 6,118,047) disclose the use of a tryptophan-insensitive α -domain of anthranilate synthase from C28 maize in a transgene to prepare transgenic maize plants (*Zea mays*) exhibiting elevated levels of free tryptophan in the seed(s).

10 Although it is possible to select for 5-MT resistance in certain cell cultures and plants, this characteristic does not necessarily correlate with the overproduction of free tryptophan in whole plants. Additionally, plants regenerated from 5-MT resistant lines frequently do not express an altered form of the enzyme. Nor is it predictable that this characteristic will be stable over a
15 period of time and will be passed along as a heritable trait.

Anthranilate synthase has also been partially purified from crude extracts of cell cultures of higher plants (Hankins et al., Plant Physiol., 57, 101 (1976); Widholm, Biochim. Biophys. Acta, 320, 217 (1973)). However, it was found to be very unstable. Thus, there is a need to provide plants with a source of
20 anthranilate synthase that can increase the tryptophan content of plants.

Summary of the Invention

The present invention provides nucleic acids encoding an anthranilate synthase (AS) that can be used to generate transgenic plants. When such
25 anthranilate synthase nucleic acids are expressed in a transgenic plant, elevated levels of tryptophan can be achieved within the cells of the plant. In one embodiment, the invention is directed to DNA molecules that encode a monomeric anthranilate synthase, where such a monomeric anthranilate synthase is a natural or genetically engineered chimeric fusion of the α - and β -domains of an anthranilate
30 synthase. The anthranilate synthase gene from a few species (e.g., some bacteria and other microbes) naturally gives rise to a monomeric anthranilate synthase that constitutes a single polypeptide chain. However, most species have a heterotetrameric anthranilate synthase composed of two α and two β domains found

on separate subunits. The invention also contemplates formation of chimeric anthranilate synthase fusion proteins comprising any anthranilate synthase α -domain linked to any β -domain.

In general, the sequence identity of naturally occurring monomeric anthranilate synthases with most plant anthranilate synthases is quite low. However, according to the invention, such monomeric anthranilate synthases can provide high levels of tryptophan when expressed in a plant, despite a low sequence identity with the plant's endogenous anthranilate synthase enzyme. Accordingly, the invention provides monomeric anthranilate synthases that can have divergent sequences and that are capable of efficiently providing high levels of tryptophan in a plant host. For example, transgenic soybean plants containing the monomeric *Agrobacterium tumefaciens* anthranilate synthase can produce from up to about 10,000 to about 12,000 ppm tryptophan in seeds, with average trp levels ranging up to about 7,000 to about 8,000 ppm. In contrast, non-transgenic soybean plants normally have up to only about 100 to about 200 ppm tryptophan in seeds.

Accordingly, the invention provides an isolated DNA sequence encoding a monomeric anthranilate synthase, wherein the monomeric anthranilate synthase has an anthranilate α -domain and an anthranilate β -domain and wherein the monomeric anthranilate synthase is expressed in a plant. Such expression can elevate the level of L-tryptophan in the plant.

The monomeric anthranilate synthase can be naturally monomeric. Examples of organisms from which naturally monomeric anthranilate synthase nucleic acids may be isolated, include but are not limited to organisms such as *Agrobacterium tumefaciens*, *Rhizobium meliloti* (e.g., Genbank Accession No. GI 95177), *Mesorhizobium loti* (e.g., Genbank Accession No. GI 13472468), *Brucella melitensis* (e.g., Genbank Accession No. GI 17982357), *Nostoc sp.* PCC7120 (e.g., Genbank Accession Nos. GI 17227910 or GI 17230725), *Azospirillum brasilense* (e.g., Genbank Accession No. GI 1174156) and *Anabaena* M22983 (e.g., Genbank Accession No. GI 152445). In some embodiments, the isolated DNA encodes an *Agrobacterium tumefaciens* anthranilate synthase having, for example, an amino acid sequence having SEQ ID NO:4 or a nucleotide sequence having any one of SEQ ID NO:1 or 75.

Alternatively, the monomeric anthranilate synthase can be a fusion of any available anthranilate synthase α and β domain. Such α and β domains can be derived from *Zea mays*, *Ruta graveolens*, *Sulfolobus solfataricus*, *Salmonella typhimurium*, *Serratia marcescens*, *Escherichia coli*, *Agrobacterium tumefaciens*,
5 *Arabidopsis thaliana*, *Rhizobium meliloti* (e.g., Genbank Accession No. GI95177), *Mesorhizobium loti* (e.g., Genbank Accession No. GI 13472468), *Brucella melitensis* (e.g., Genbank Accession No. GI 17982357), *Nostoc sp.* PCC7120 (e.g., Genbank Accession No. GI 17227910 or GI 17230725), *Azospirillum brasilense* (e.g., Genbank Accession No. GI 1174156) and *Anabaena* M22983 (e.g., Genbank
10 Accession No. GI 152445)), soybean, rice, cotton, wheat, tobacco or any gene encoding a subunit or domain of anthranilate synthase. For example, nucleic acids encoding such an α or β domain can be obtained by using the sequence information in any of SEQ ID NO:1 -70, 75-103.

In another embodiment, the invention provides an isolated DNA encoding an
15 α domain of anthranilate synthase from *Zea mays* that comprises SEQ ID NO:5, or SEQ ID NO:66. Such an isolated DNA can have nucleotide sequence SEQ ID NO:2, 67 or 68. The isolated DNA can be operably linked to a promoter and, when expressed in a plant can provide elevated levels of L-tryptophan in the plant.

The isolated DNA can also encode a mutant anthranilate synthase, or a
20 mutant anthranilate synthase domain. Such a mutant anthranilate synthase, or domain thereof, can have one or more mutations. As is known to one of skill in the art, mutations can be silent, can give rise to variant gene products having enzymatic activity similar to wild type or can give rise to derivative gene products that have altered enzymatic activity. The invention contemplates all such mutations.

25 The mutated isolated DNA can be generated from a wild type anthranilate synthase nucleic acid either *in vitro* or *in vivo* and can encode, for example, one or more amino acid substitutions, deletions or insertions. Mutant isolated DNAs that generate a mutant anthranilate synthase having increased activity, greater stability, or less sensitivity to feedback inhibition by tryptophan or tryptophan analogs are desirable. In one embodiment, the anthranilate synthase, or a domain thereof, is
30 resistant to inhibition by endogenous L-tryptophan or by tryptophan analogs. For example, the anthranilate synthase can have one or more mutations in the tryptophan-binding pocket or elsewhere that reduces the sensitivity of the

anthranilate synthase, or the domain thereof, to tryptophan inhibition. Among the amino acid residues contemplated for mutation are residues, for example, at about positions 48, 51, 52, 293 and 298. For example, the mutation can be:

- a) at about position 48 replace Val with Phe;
- 5 b) at about position 48 replace Val with Tyr;
- c) at about position 51 replace Ser with Phe;
- d) at about position 51 replace Ser with Cys;
- e) at about position 52 replace Asn with Phe;
- f) at about position 293 replace Pro with Ala;
- 10 g) at about position 293 replace Pro with Gly; or
- h) at about position 298 replace Phe with Trp;

wherein the position of the mutation is determined by alignment of the amino acid sequence of the selected anthranilate synthase with an *Agrobacterium tumefaciens* anthranilate synthase amino acid sequence. Examples of
15 anthranilate synthases having such mutations include those with SEQ ID NO:58-65, 69, 70, 84-94.

The isolated DNA can encode other elements and functions. Any element or function contemplated by one of skill in the art can be included. For example, the isolated DNA can also include a promoter that can function in a
20 plant cell that is operably linked to the DNA encoding the anthranilate synthase. The isolated DNA can further encode a plastid transit peptide. The isolated DNA can also encode a selectable marker or a reporter gene. Such a selectable marker gene can impart herbicide resistance to cells of said plant, high protein content, high oil content, high lysine content, high isoleucine content, high
25 tocopherol content and the like. The DNA sequence can also comprise a sequence encoding one or more of the insecticidal proteins derived from *Bacillus thuringiensis*.

The invention further provides vectors comprising an isolated DNA of the invention. Such vectors can be used to express anthranilate synthase
30 polypeptides in prokaryotic and eukaryotic cells, to transform plant cells and to generate transgenic plants.

The invention also provides a transgenic plant comprising an isolated DNA of the invention. Expression of these isolated DNAs in the transgenic

plant can result in an elevated level of L-tryptophan, preferably free L-tryptophan, in the transgenic plant, e.g., in the seeds or other parts of the plant. The level is increased above the level of L-tryptophan in the cells of a plant that differ from the cells of the transgenic soybean plant by the absence of the DNA, e.g., the corresponding untransformed cells or an untransformed plant with the same genetic background. The DNA is preferably heritable in that it is preferably transmitted through a complete normal sexual cycle of the fertile plant to its progeny and to further generations.

Transgenic plants that can have such an isolated DNA include dicotyledonous plants (dicots), for example, soybean or canola. Alternatively, the transgenic plants can be monocotyledonous plants (monocots), for example, maize, rice, wheat, barley or sorghum.

The invention also provides a seed of any of the transgenic plants containing any of the isolated DNAs, anthranilate synthase polypeptides, transgenes or vectors of the invention.

The invention further provides an animal feed or human food that contains at least a portion of a plant having an isolated DNA of the invention. Portions of plants that can be included in the animal feed or human food include, for example, seeds, leaves, stems, roots, tubers, or fruits. Desirable portions of plants have increased levels of tryptophan provided by expression of an anthranilate synthase encoded by an isolated DNA of the invention.

The invention further provides a method for altering, preferably increasing, the tryptophan content of a plant (dicot or a monocot) by introducing an isolated DNA of the invention into regenerable cells of the plant. The DNA sequence is preferably operably linked to at least one promoter operable in the plant cells. The transformed cells are identified or selected, and then regenerated to yield a plant comprising cells that can express a functional anthranilate synthase polypeptide. In some embodiments, the DNA encoding the anthranilate synthase, or domain thereof, is a mutant DNA. The introduced DNA is preferably heritable and the plant is preferably a fertile plant. For example, the introduced DNA preferably can be passed by a complete sexual cycle to progeny plants, and can impart the high tryptophan phenotype to subsequent generations of progeny.

The anthranilate synthase-encoding DNAs, are preferably incorporated into vectors or "transgenes" that can also include DNA sequences encoding transit peptides, such as plastid transit peptides, and selectable marker or reporter genes, operably linked to one or more promoters that are functional in cells of the target plant. The promoter can be, for example, an inducible promoter, a tissue specific promoter, a strong promoter or a weak promoter. Other transcription or translation regulatory elements, e.g., enhancers or terminators, can also be functionally linked to the anthranilate synthase-encoding DNA segment.

Cells in suspension culture or as embryos, intact tissues or organs can be transformed by a wide variety of transformation techniques, for example, by microprojectile bombardment, electroporation and *Agrobacterium tumefaciens*-mediated transformation, and other procedures available to the art.

Thus, the cells of the transformed plant comprise a native anthranilate synthase gene and a transgene or other DNA segment encoding an exogenous anthranilate synthase. The expression of the exogenous anthranilate synthase in the cells of the plant can lead to increased levels of tryptophan and its secondary metabolites. In some embodiments, such expression confers tolerance to an amount of endogenous L-tryptophan analogue, for example, so that at least about 10% more anthranilate synthase activity is present than in a plant cell having a wild type or tryptophan-sensitive anthranilate synthase.

The invention also provides a method for altering the tryptophan content in a plant comprising: (a) introducing into regenerable cells of a plant a transgene comprising an isolated DNA encoding an anthranilate synthase domain and a plastid transit peptide, operably linked to a promoter functional in the plant cell to yield transformed cells; and (b) regenerating a transformed plant from said transformed plant cells wherein the cells of the plant express the anthranilate synthase domain encoded by the isolated DNA in an amount effective to increase the tryptophan content in said plant relative to the tryptophan content in an untransformed plant of the same genetic background. The domain can be an anthranilate synthase α -domain. The anthranilate synthase domain can have one or more mutations, for example, mutations that reduce the sensitivity of the domain to tryptophan inhibition. Such mutations can be, for example, in the

tryptophan-binding pocket. Such a domain can be, for example, an anthranilate synthase domain from *Agrobacterium tumefaciens*, *Anabaena* M22983, *Arabidopsis thaliana*, *Azospirillum brasilense*, *Brucella melitensis*, *Escherichia coli*, *Euglena gracilis*, *Mesorhizobium loti*, *Nostoc* sp. PCC7120, *Rhizobium meliloti*, *Ruta graveolens*, *Rhodopseudomonas palustris*, *Salmonella typhimurium*, *Serratia marcescens*, *Sulfolobus solfataricus*, soybean, rice, cotton or *Zea mays*. *Ruta graveolens* has its own chloroplast transport sequence that may be used with the anthranilate synthase transgene. Accordingly, one of skill in the art may not need to add a plastid transport sequence when using a *Ruta graveolens* DNA.

The present invention also provides novel isolated and purified DNA molecules comprising a DNA encoding a monomeric anthranilate synthase, or a domain thereof. Such an anthranilate synthase DNA can provide high levels of tryptophan when expressed within a plant. In some embodiments, the anthranilate synthase is substantially resistant to inhibition by free L-tryptophan or an analog thereof. Examples of novel DNA sequences contemplated by the invention include but are not limited to DNA molecules isolated from *Agrobacterium tumefaciens*, *Anabaena* M22983, *Arabidopsis thaliana*, *Azospirillum brasilense*, *Brucella melitensis*, *Escherichia coli*, *Euglena gracilis*, *Mesorhizobium loti*, *Nostoc* sp. PCC7120, *Rhizobium meliloti*, *Ruta graveolens*, *Rhodopseudomonas palustris*, *Salmonella typhimurium*, *Serratia marcescens*, *Sulfolobus solfataricus*, or *Zea mays* (maize) or other such anthranilate synthases.

These DNA sequences include synthetic or naturally-occurring monomeric forms of anthranilate synthase that have the α -domain of anthranilate synthase linked to at least one other anthranilate synthase domain on a single polypeptide chain. The monomeric anthranilate synthase can, for example, be a fusion of an anthranilate synthase α or β domain. Such an anthranilate synthase α or β domain can be derived from *Agrobacterium tumefaciens*, *Anabaena* M22983, *Arabidopsis thaliana*, *Azospirillum brasilense*, *Brucella melitensis*, *Escherichia coli*, *Euglena gracilis*, *Mesorhizobium loti*, *Nostoc* sp. PCC7120, *Rhizobium meliloti*, *Ruta graveolens*, *Rhodopseudomonas palustris*, *Salmonella typhimurium*, *Serratia marcescens*, *Sulfolobus solfataricus*, soybean, rice, cotton,

wheat, tobacco or *Zea mays* (maize) or any gene encoding a subunit or domain of anthranilate synthase. Such anthranilate synthases and domains thereof are also exemplified herein by the anthranilate synthase nucleic acids isolated from *Agrobacterium tumefaciens*, (SEQ ID NO:1, 75, 84-94), *Zea mays*, (SEQ ID NO:2, 67, 68, 96), *Ruta graveolens* (SEQ ID NO:3), *Anabaena* M22983, *Arabidopsis thaliana* (SEQ ID NO:45), *Azospirillum brasilense* (SEQ ID NO:78), *Brucella melitensis* (SEQ ID NO:79), *Mesorhizobium loti* (SEQ ID NO:77), *Nostoc sp.* PCC7120 (SEQ ID NO:80 or 81), *Rhizobium meliloti* (SEQ ID NO:7), *Rhodopseudomonas palustris* (SEQ ID NO:57), *Sulfolobus solfataricus* (SEQ ID NO:8), rice (SEQ ID NO:94 or 95), wheat (SEQ ID NO:97), or tobacco (SEQ ID NO:98). These nucleotide sequences encode anthranilate synthases or α -domains thereof from *Agrobacterium tumefaciens* (SEQ ID NO:4, 58-65, 69, 70,); *Zea mays* (SEQ ID NO:5, 66 or 101) and *Ruta graveolens* (SEQ ID NO:6), *Anabaena* M22983, *Azospirillum brasilense* (SEQ ID NO:78), *Brucella melitensis* (SEQ ID NO:79), *Mesorhizobium loti* (SEQ ID NO:77), *Nostoc sp.* PCC7120 (SEQ ID NO:80 or 81), *Rhizobium meliloti* (SEQ ID NO:7 or 43), *Rhodopseudomonas palustris* (SEQ ID NO:57 or 82), *Sulfolobus solfataricus* (SEQ ID NO:8 or 44), rice (SEQ ID NO:99 or 100), wheat (SEQ ID NO:102), or tobacco (SEQ ID NO:103).

The invention also provides an isolated DNA molecule comprising a DNA sequence encoding an *Agrobacterium tumefaciens* anthranilate synthase or a domain thereof having enzymatic activity. Such a DNA molecule can encode an anthranilate synthase having SEQ ID NO:4, 58-65, 69 or 70, a domain or variant thereof having anthranilate synthase activity. The DNA molecule can also have a sequence comprising SEQ ID NO:1, 75, 84-94, or a domain or variant thereof. Coding regions of any DNA molecule provided herein can also be optimized for expression in a selected organism, for example, a selected plant or microbe. An example of a DNA molecule having optimized codon usage for a selected plant is an *Agrobacterium tumefaciens* anthranilate synthase DNA molecule having SEQ ID NO:75.

The invention also provides an isolated and purified DNA molecule comprising a DNA sequence encoding a *Zea mays* anthranilate synthase domain. Such a DNA molecule can encode an anthranilate synthase domain having SEQ

ID NO:5, 66 or a variant or derivative thereof having anthranilate synthase activity. The DNA molecule can also have a sequence comprising SEQ ID NO:2, 67 or 68, or a domain or variant thereof.

- The invention further provides an isolated DNA molecule of at least 8
5 nucleotides that hybridizes to the complement of a DNA molecule comprising any one of SEQ ID NO:1, 75 or 84-94 under stringent conditions. Such a DNA molecule can be a probe or a primer, for example, a nucleic acid having any one of SEQ ID NO:9-42 or 47-56. Alternatively, the DNA it can include up to an entire coding region for a selected anthranilate synthase, or a domain thereof.
10 Such a DNA can also include a DNA sequence encoding a promoter operable in plant cells and/or a DNA sequence encoding a plastid transit peptide. The invention further contemplates vectors for transformation and expression of these types of DNA molecules in plants and/or microbes.

- Functional anthranilate synthase DNA sequences and functional
15 anthranilate synthase polypeptides that exhibit 50%, preferably 60%, more preferably 70%, even more preferably 80%, most preferably 90%, e.g., 95% to 99%, sequence identity to the DNA sequences and amino acid sequences explicitly described herein are also within the scope of the invention. For example, 85% identity means that 85% of the amino acids are identical when the
20 two sequences are aligned for maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred.

- Alternatively and preferably, two polypeptide sequences are homologous, as this term is used herein, if they have an alignment score of more than 5 (in
25 standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. See Dayhoff, M.O., in Atlas of Protein Sequence and Structure, 1972, volume 5, National Biomedical Research Foundation, pp. 101-110, and Supplement 2 to this volume, pp. 1-10. The two sequences or parts thereof are more preferably homologous if their amino acids
30 are greater than or equal to 50% identical when optimally aligned using the ALIGN program.

The invention further provides expression vectors for generating a transgenic plant with high seed levels of tryptophan comprising an isolated DNA

sequence encoding a monomeric anthranilate synthase comprising an anthranilate synthase α -domain linked to an anthranilate synthase β -domain and a plastid transit peptide, operably linked to a promoter functional in a plant cell. Such a monomeric anthranilate synthase can, for example, be an *Agrobacterium tumefaciens*, *Rhizobium meliloti*, *Mesorhizobium loti*, *Brucella melitensis*,
5 *Nostoc sp.* PCC7120, *Azospirillum brasilense* or *Anabaena* M22983 anthranilate synthase. The monomeric anthranilate synthase can also be a fusion of anthranilate synthase α and β domains derived from *Agrobacterium tumefaciens*, *Anabaena* M22983, *Arabidopsis thaliana*, *Azospirillum brasilense*, *Brucella*
10 *melitensis*, *Mesorhizobium loti*, *Nostoc sp.* PCC7120, *Rhizobium meliloti*, *Rhodospseudomonas palustris*, *Ruta graveolens*, *Sulfolobus solfataricus*, *Salmonella typhimurium*, *Serratia marcescens*, soybean, rice, cotton, wheat, tobacco *Zea mays*, or any gene encoding a subunit or domain of anthranilate synthase.

15 The transmission of the isolated and purified anthranilate synthase DNA providing increased levels of tryptophan can be evaluated at a molecular level, e.g., Southern or Northern blot analysis, PCR-based methodologies, the biochemical or immunological detection of anthranilate synthase, or by phenotypic analyses, i.e., whether cells of the transformed progeny can grow in
20 the presence of an amount of an amino acid analog of tryptophan that inhibits the growth of untransformed plant cells.

The invention also provides a method of producing anthranilate synthase in a prokaryotic or eukaryotic host cell, such as a yeast, insect cell, or bacterium, which can be cultured, preferably on a commercial scale. The method includes
25 the steps of introducing a transgene comprising a DNA segment encoding an anthranilate synthase, or a domain thereof, such as a monomeric anthranilate synthase, comprising at least the α and β anthranilate synthase domains, or functional variant thereof, into a host cell and expressing anthranilate synthase in the host cell so as to yield functional anthranilate synthase or domain thereof. A
30 transgene generally includes transcription and translation regulatory elements, e.g., a promoter, functional in host cell, either of eukaryotic or prokaryotic origin. Preferably, the transgene is introduced into a prokaryotic cell, such as *Escherichia coli*, or a eukaryotic cell, such as a yeast or insect cell, that is known

to be useful for production of recombinant proteins. Culturing the transformed cells can lead to enhanced production of tryptophan and its derivatives, which can be recovered from the cells or from the culture media. Accumulation of tryptophan may also lead to the increased production of secondary metabolites in microbes and plants, for example, indole containing metabolites such as simple indoles, indole conjugates, indole alkaloids, indole phytoalexins and indole glucosinates in plants.

Anthranilate synthases insensitive to tryptophan have the potential to increase a variety of chorismate-derived metabolites, including those derived from phenylalanine due to the stimulation of phenylalanine synthesis by tryptophan via chorismate mutase. See Siehl, D. The biosynthesis of tryptophan, tyrosine, and phenylalanine from chorismate in *Plant Amino Acids: Biochemistry and Biotechnology*, ed. BK Singh, pp 171-204. Other chorismate-derived metabolites that may increase when feedback insensitive anthranilate synthase s are present include phenylpropanoids, flavonoids, and isoflavonoids, as well as those derived from anthranilate, such as indole, indole alkaloids, and indole glucosinolates. Many of these compounds are important plant hormones, plant defense compounds, chemopreventive agents of various health conditions, and/or pharmacologically active compounds. The range of these compounds whose synthesis might be increased by expression of anthranilate synthase depends on the organism in which the anthranilate synthase is expressed. The invention contemplates synthesis of tryptophan and other useful compounds in a variety of prokaryotic and eukaryotic cells or organisms, including plant cells, microbes, fungi, yeast, bacteria, insect cells, and mammalian cells.

Hence, the invention provides a method for producing tryptophan comprising: culturing a prokaryotic or eukaryotic host cell comprising an isolated DNA under conditions sufficient to express a monomeric anthranilate synthase encoded by the isolated DNA, wherein the monomeric anthranilate synthase comprises an anthranilate synthase α domain and a anthranilate synthase β domain, and wherein the conditions sufficient to express a monomeric anthranilate synthase comprise nutrients and precursors sufficient for the host cell to synthesize tryptophan utilizing the monomeric anthranilate synthase.

Examples of useful compounds that may be generated upon expression in a variety of host cells and/or organisms include indole acetic acid and other auxins, isoflavonoid compounds important to cardiovascular health found in soy, volatile indole compounds which act as signals to natural enemies of herbivorous insects in maize, anticarcinogens such as indole glucosinolates (indole-3-carbinol) found in the Cruciferae plant family, as well as indole alkaloids such as ergot compounds produced by certain species of fungi. (Barnes et al., Adv Exp Med Biol, 401, 87 (1996); Frey et al., Proc Natl Acad Sci, 97, 14801 (2000); Muller et al., Biol Chem, 381, 679 (2000); Mantegani et al., Farmacol, 54, 288 (1999); Zeligs, J Med Food, 1, 67 (1998); Mash et al., Ann NY Acad Sci, 844, 274 (1998); Melanson et al., Proc Natl Acad Sci, 94, 13345 (1997); Broadbent et al., Curr Med Chem, 5, 469 (1998)).

The present invention also provides an isolated and purified DNA molecule of at least seven nucleotide bases that hybridizes under moderate, and preferably, high stringency conditions to the complement of an anthranilate synthase encoding DNA molecule. Such isolated and purified DNA molecules comprise novel DNA segments encoding anthranilate synthase or a domain or mutant thereof. The mutant DNA can encode an anthranilate synthase that is substantially resistant to inhibition by free L-tryptophan or an amino acid analog of tryptophan. Such anthranilate synthase DNA molecules can hybridize, for example, to an *Agrobacterium tumefaciens*, *Rhodopseudomonas palustris* or *Ruta graveolens* anthranilate synthase, or an α -domain thereof, including functional mutants thereof. When these DNA molecules encode a functional anthranilate synthase or an anthranilate synthase domain, they are termed “variants” of the primary DNA molecules encoding anthranilate synthase, anthranilate synthase domains or mutants thereof. Shorter DNA molecules or oligonucleotides can be employed as primers for amplification of target DNA sequences by PCR, or as intermediates in the synthesis of full-length genes.

Also provided is a hybridization probe comprising a novel isolated and purified DNA segment of at least seven nucleotide bases, which is detectably labeled or which can bind to a detectable label, which DNA segment hybridizes under moderate or, preferably, high stringency conditions to the non-coding strand of a DNA molecule comprising a DNA segment encoding an anthranilate

synthase such as a monomeric anthranilate synthase, or a domain thereof, such as the α -domain, including functional mutants thereof, that are substantially resistant to inhibition by an amino acid analog of tryptophan. Moderate and stringent hybridization conditions are well known to the art, see, for example

5 sections 0.47-9.51 of Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edition (1989); *see also*, Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, 3rd Edition (January 15, 2001). For example, stringent conditions are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate (SSC); 0.1%

10 sodium lauryl sulfate (SDS) at 50°C, or (2) employ a denaturing agent such as formamide during hybridization, e.g., 50% formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is use of 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium

15 citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 μ g/ml), 0.1% sodium dodecylsulfate (SDS), and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

20 **Brief Description of the Figures**

Figure 1 is a restriction map of pMON61600.

Figure 2 depicts the translated sequence of the *Agrobacterium tumefaciens* anthranilate synthase DNA sequence (upper sequence) (SEQ ID NO:4) and the translated sequence of the anthranilate synthase DNA sequence

25 from *Rhizobium meliloti* (lower sequence) (SEQ ID NO:7).

Figure 3 is a restriction map of pMON34692.

Figure 4 is a restriction map of pMON34697.

Figure 5 is a restriction map of pMON34705.

Figure 6 (A-B) depicts an anthranilate synthase amino acid sequence

30 alignment comparing the *Agrobacterium tumefaciens* α -domain sequence (SEQ ID NO:4) and the *Sulfolobus solfataricus* α -domain sequence (SEQ ID NO:8).

Figure 7 (A-B) depicts the sequences of the 34 primers (SEQ ID NOs 9-42) used to mutate SEQ ID NO:1. The mutated codons are underlined and the changed bases are in lower case.

Figure 8 depicts a restriction map of plasmid pMON13773.

5 Figure 9 depicts a restriction map of plasmid pMON58044.

Figure 10 depicts a restriction map of plasmid pMON53084.

Figure 11 depicts a restriction map of plasmid pMON58045.

Figure 12 depicts a restriction map of plasmid pMON58046.

Figure 13 depicts a restriction map of plasmid pMON38207.

10 Figure 14 depicts a restriction map of plasmid pMON58030.

Figure 15 depicts a restriction map of plasmid pMON58006.

Figure 16 depicts a restriction map of plasmid pMON58041.

Figure 17 depicts a restriction map of plasmid pMON58028.

Figure 18 depicts a restriction map of plasmid pMON58042.

15 Figure 19 depicts a restriction map of plasmid pMON58029.

Figure 20 depicts a restriction map of plasmid pMON58043.

Figure 21 (A-D) depicts a multiple sequence alignment of monomeric "TrpEG" anthranilate synthases having SEQ ID NO:4 and 43 (derived from *Agrobacterium tumefaciens* and *Rhizobium meliloti*, respectively) with the TrpE (α) and TrpG (β) domains of heterotetrameric anthranilate synthases from *Sulfolobus solfataricus* (SEQ ID NO:44) and *Arabidopsis thaliana* (SEQ ID NO:45). Linker regions are underlined.

Figure 22 is a restriction map of plasmid pMON52214.

Figure 23 is a restriction map of plasmid pMON53901.

25 Figure 24 is a restriction map of plasmid pMON39324.

Figure 25 is a restriction map of plasmid pMON39322.

Figure 26 is a restriction map of plasmid pMON39325.

Figure 27 is a graph depicting free tryptophan levels in soybean seeds transformed with pMON39325. There were five observations from each event.

30 NT represents non-transgenic soybean seed.

Figure 28 is a restriction map of plasmid pMON25997.

Figure 29 is a restriction map of plasmid pMON62000.

Figure 30 depicts the sequence of the truncated *trpE* gene of *Escherichia coli* EMG2 (K-12 wt F+) (SEQ ID NO:46). The first 30bp and the last 150bp of this *trpE* nucleic acid are connected by an EcoR1 restriction site. The beginning of the *trpG* gene follows the *trpE* stop codon.

5 Figure 31 schematically depicts construction of the in-frame deletion in the *E. coli trpE* gene.

Figure 32 (A-C) depicts the DNA (SEQ ID NO:1) and amino acid (SEQ ID NO:4) sequences of the α -domain of the anthranilate synthase gene isolated from *Agrobacterium tumefaciens*.

10 Figure 33 (A-C) depicts the DNA (SEQ ID NO:2) sequence of the α -domain of the anthranilate synthase gene isolated from *Zea mays*. Figure 33 (D) depicts the amino acid (SEQ ID NO:5) sequence of the α -domain of the anthranilate synthase gene isolated from *Zea mays*.

Figure 34 is a restriction map of plasmid pMON58120.

15 Figure 35 (A-E) provides a sequence comparison of anthranilate synthase amino acid sequences from *Agrobacterium tumefaciens* (AgrTu_15889565) (SEQ ID NO:4), *Rhizobium meliloti* (RhiMe_136328) (SEQ ID NO:7), *Mesorhizobium loti* (MesLo_13472468) (SEQ ID NO:77), *Azospirillum brasilense* (AzoBr_1717765) (SEQ ID NO:78), *Brucella melitensis* (BruMe_17986732) (SEQ ID NO:79), *Nostoc sp.* (Nostoc_17227910) (SEQ ID NO:80), *Nostoc sp.* (Nostoc_17230725) (SEQ ID NO:81), and *Rhodospseudomonas palustris* (RhoPa_TrpEG) (SEQ ID NO:82).

Figure 36 (A-B) provides an optimized nucleotide sequence for *Agrobacterium tumefaciens* anthranilate synthase (SEQ ID NO:75).

25 Figure 37 (A-C) provides an alignment of the wild type (top strand) and optimized (bottom strand) *Agrobacterium tumefaciens* anthranilate synthase nucleotide sequences (SEQ ID NO:1 and 75). These two sequences are 94% identical.

30

Detailed Description of the Invention

The present invention provides isolated DNAs, vectors, host cells and transgenic plants comprising an isolated nucleic acid encoding an anthranilate synthase capable of providing high levels of tryptophan upon expression within

the plant. In one embodiment, the isolated nucleic acid encodes a monomeric anthranilate synthase (AS). In other embodiments, the isolated nucleic acid encodes an anthranilate synthase, or a domain thereof, that is substantially resistant to inhibition by free L-tryptophan or an amino acid analog of
5 tryptophan. Expression of the anthranilate synthase, or domain thereof, elevates the level of tryptophan, e.g., free tryptophan in the seed, over the level present in the plant absent such expression.

Methods are also provided for producing transgenic plants having nucleic acids associated with increased anthranilate synthase activity, and producing
10 cultured cells, plant tissues, plants, plant parts and seeds that produce high levels of tryptophan. Such transgenic plants can preferably sexually transmit the ability to produce high levels of tryptophan to their progeny. Also described are methods for producing isolated DNAs encoding mutant anthranilate synthases, and cell culture selection techniques to select for novel genotypes that
15 overproduce tryptophan and/or are resistant to tryptophan analogs. For example, to produce soybean lines capable of producing high levels of tryptophan, transgenic soybean cells that contain at least one of the isolated DNAs of the invention, are prepared and characterized, then regenerated into plants. Some of the isolated DNAs are resistant to growth inhibition by the tryptophan analog.
20 The methods provided in the present invention may also be used to produce increased levels of free tryptophan in dicot plants, such as other legumes, as well as in monocots, such as the cereal grains.

Definitions

25 As used herein, "altered" levels of tryptophan in a transformed plant, plant tissue, plant part or plant cell are levels which are greater or lesser than the levels found in the corresponding untransformed plant, plant tissue, plant part or plant cell.

As used herein, a " α -domain" is a portion of an enzyme or enzymatic
30 complex that binds chorismate and eliminates the enolpyruvate side chain. Such an α -domain can be encoded by a TrpE gene. In some instances, the α -domain is a single polypeptide that functions only to bind chorismate and to eliminate the enolpyruvate side chain from chorismate. In other instances, the α -domain is

part of a larger polypeptide that can carry out other enzymatic functions in addition to binding chorismate and eliminating the enolpyruvate side chain from chorismate.

The term " β -domain" refers to a portion of an enzyme or enzymatic complex that transfers an amino group from glutamine to the position on the chorismate ring that resides between the carboxylate and the enolpyruvate moieties. Such a β -domain can be encoded by a TrpG gene. In some instances, the β -domain is a single polypeptide that functions only to transfer an amino group from glutamine to the position on the chorismate ring that resides between the carboxylate and the enolpyruvate moieties. In other instances, the β -domain is part of a larger polypeptide that can carry out other enzymatic functions in addition to transferring an amino group from glutamine to the position on the chorismate ring that resides between the carboxylate and the enolpyruvate moieties.

As used herein, "an amino acid analog of tryptophan" is an amino acid that is structurally related to tryptophan and that can bind to the tryptophan-binding site in a wild type anthranilate synthase. These analogs include, but are not limited to, 6-methylantranilate, 5-methyltryptophan, 4-methyltryptophan, 5-fluorotryptophan, 5-hydroxytryptophan, 7-azatryptophan, 3 β -indoleacrylic acid, 3-methylantranilic acid, and the like.

The term "consists essentially of" as used with respect to the present DNA molecules, sequences or segments is defined to mean that a major portion of the DNA molecule, sequence or segment encodes an anthranilate synthase. Unless otherwise indicated, the DNA molecule, sequence or segment generally does not encode proteins other than an anthranilate synthase.

The term "complementary to" is used herein to mean that the sequence of a nucleic acid strand could hybridize to all, or a portion, of a reference polynucleotide sequence. For illustration, the nucleotide sequence "TATAC" has 100% identity to a reference sequence 5'-TATAC-3' but is 100% complementary to a reference sequence 5'-GTATA-3'.

As used herein, an "exogenous" anthranilate synthase is an anthranilate synthase that is encoded by an isolated DNA that has been introduced into a host cell, and that is preferably not identical to any DNA sequence present in the cell

in its native, untransformed state. An "endogenous" or "native" anthranilate synthase is an anthranilate synthase that is naturally present in a host cell or organism.

As used herein, "increased" or "elevated" levels of free L-tryptophan in a
5 plant cell, plant tissue, plant part or plant are levels that are about 2 to 200 times, preferably about 5 to 150 times, and more preferably about 10-100 times, the levels found in an untransformed plant cell, plant tissue, plant part or plant, i.e., one where the genome has not been altered by the presence of an exogenous anthranilate synthase nucleic acid or domain thereof. For example, the levels of
10 free L-tryptophan in a transformed plant seed are compared with those in an untransformed plant seed ("the starting material").

DNA molecules encoding an anthranilate synthase, and DNA molecules encoding a transit peptide or marker/reporter gene are "isolated" in that they were taken from their natural source and are no longer within the cell where they
15 normally exist. Such isolated DNA molecules may have been at least partially prepared or manipulated *in vitro*, e.g., isolated from a cell in which they are normally found, purified, and amplified. Such isolated DNA molecules can also be "recombinant" in that they have been combined with exogenous DNA molecules or segments. For example, a recombinant DNA can be an isolated
20 DNA that is operably linked to an exogenous promoter, or to a promoter that is endogenous to the host cell.

As used herein with respect to anthranilate synthase, the term "monomeric" means that two or more anthranilate synthase domains are incorporated in a functional manner into a single polypeptide chain. The
25 monomeric anthranilate synthase may be assembled *in vivo* into a dimeric form. Monomeric anthranilate synthase nucleic acids and polypeptides can be isolated from various organisms such as *Agrobacterium tumefaciens*, *Anabaena* M22983, *Azospirillum brasilense*, *Brucella melitensis*, *Euglena gracilis*, *Mesorhizobium loti*, *Nostoc sp.* PCC7120 or *Rhizobium meliloti*. Alternatively, monomeric
30 anthranilate synthase nucleic acids and polypeptides can be constructed from a combination of domains selected from any convenient monomeric or multimeric anthranilate synthase gene. Such organisms include, for example, *Agrobacterium tumefaciens*, *Anabaena* M22983, *Arabidopsis thaliana*,

Azospirillum brasilense, *Brucella melitensis*, *Mesorhizobium loti*, *Nostoc sp.* PCC7120, *Rhizobium meliloti*, *Rhodopseudomonas palustris*, *Ruta graveolens*, *Sulfolobus solfataricus*, *Salmonella typhimurium*, *Serratia marcescens*, soybean, rice, cotton *Zea mays*, or any gene encoding a subunit or domain of anthranilate synthase. Nucleic acids encoding the selected domains can be linked recombinationally. For example, a nucleic acid encoding the C-terminus of an α -domain can be linked to a nucleic acid encoding the N-terminus of the β -domain, or vice versa, by forming a phosphodiester bond. As an alternative, such single domain polypeptides can be linked chemically. For example, the α -domain can be linked via its C-terminus to the N-terminus of the β -domain, or vice versa, by forming a peptide bond.

As used herein, a "native" gene means a gene that has not been changed *in vitro*, i.e., a "wild-type" gene that has not been mutated *in vitro*.

The term "plastid" refers to the class of plant cell organelles that includes amyloplasts, chloroplasts, chromoplasts, elaioplasts, coplasts, etioplasts, leucoplasts, and proplastids. These organelles are self-replicating, and contain what is commonly referred to as a "chloroplast genome," a circular DNA molecule that ranges in size from about 120 to about 217 kb, depending upon the plant species, and which usually contains an inverted repeat region.

As used herein, "polypeptide" means a continuous chain of amino acids that are all linked together by peptide bonds, except for the N-terminal and C-terminal amino acids that have amino and carboxylate groups, respectively, and that are not linked in peptide bonds. Polypeptides can have any length and can be post-translationally modified, for example, by glycosylation or phosphorylation.

As used herein, a plant cell, plant tissue or plant that is "resistant or tolerant to inhibition by an amino acid analog of tryptophan" is a plant cell, plant tissue, or plant that retains at least about 10% more anthranilate synthase activity in the presence of an analog of L-tryptophan, than a corresponding wild type anthranilate synthase. In general, a plant cell, plant tissue, or plant that is "resistant or tolerant to inhibition by an amino acid analog of tryptophan" can grow in an amount of an amino acid analog of tryptophan that normally inhibits growth of the untransformed plant cell, plant tissue, or plant, as determined by

methodologies known to the art. For example, a homozygous backcross converted inbred plant transformed with a DNA molecule that encodes an anthranilate synthase that is substantially resistant or tolerant to inhibition by an amino acid analog of tryptophan grows in an amount of an amino acid analog of tryptophan that inhibits the growth of the corresponding, i.e., substantially isogenic, recurrent inbred plant.

As used herein, an anthranilate synthase that is "resistant or tolerant to inhibition by tryptophan or an amino acid analog of tryptophan" is an anthranilate synthase that retains greater than about 10% more activity than a corresponding "wild-type" or native susceptible anthranilate synthase, when the tolerant/resistant and wild type anthranilate synthases are exposed to equivalent amounts of tryptophan or an amino acid analog of tryptophan. Preferably the resistant or tolerant anthranilate synthase retains greater than about 20% more activity than a corresponding "wild-type" or native susceptible anthranilate synthase.

As used herein with respect to anthranilate synthase, the term "a domain thereof," includes a structural or functional segment of a full-length anthranilate synthase. A structural domain includes an identifiable structure within the anthranilate synthase. An example of a structural domain includes an alpha helix, a beta sheet, an active site, a substrate or inhibitor binding site and the like. A functional domain includes a segment of an anthranilate synthase that performs an identifiable function such as a tryptophan binding pocket, an active site or a substrate or inhibitor binding site. Functional domains of anthranilate synthase include those portions of anthranilate synthase that can catalyze one step in the biosynthetic pathway of tryptophan. For example, an α -domain is a domain that can be encoded by *trpE* and that can transfer NH_3 to chorismate and form anthranilate. A β -domain can be encoded by *trpG* and can remove an amino group from glutamine to form ammonia. Hence, a functional domain includes enzymatically active fragments and domains of an anthranilate synthase. Mutant domains of anthranilate synthase are also contemplated. Wild type anthranilate synthase nucleic acids utilized to make mutant domains include, for example, any nucleic acid encoding a domain of *Agrobacterium tumefaciens*, *Anabaena* M22983, *Arabidopsis thaliana*, *Azospirillum brasilense*, *Brucella*

melitensis, *Mesorhizobium loti*, *Nostoc* sp. PCC7120, *Rhizobium meliloti*,
Rhodopseudomonas palustris, *Ruta graveolens*, *Sulfolobus solfataricus*,
Salmonella typhimurium, *Serratia marcescens*, soybean, rice, cotton, wheat,
tobacco *Zea mays*, or any gene encoding a subunit or domain of anthranilate
5 synthase that can comprise at least one amino acid substitution in the coding
region thereof. Domains that are mutated or joined to form a monomeric
anthranilate synthase having increased tryptophan biosynthetic activity, greater
stability, reduced sensitivity to tryptophan or an analog thereof, and the like, are
of particular interest.

10

General Concepts

The present invention relates to novel nucleic acids and methods for
obtaining plants that produce elevated levels of free L-tryptophan. The
overproduction results from the introduction and expression of a nucleic acid
15 encoding anthranilate synthase, or a domain thereof. Such anthranilate synthase
nucleic acids include wild type or mutant α -domains, or monomeric forms of
anthranilate synthase. A monomeric form of anthranilate synthase comprises at
least two anthranilate synthase domains in a single polypeptide chain, e.g., an α -
domain linked to a β -domain.

20 Native plant anthranilate synthases are generally quite sensitive to
feedback inhibition by L-tryptophan and analogs thereof. Such inhibition
constitutes a key mechanism for regulating the tryptophan synthetic pathway.
Therefore, an anthranilate synthase or a domain thereof that is highly active,
more efficient or that is inhibited to a lesser extent by tryptophan or an analog
25 thereof will likely produce elevated levels of tryptophan. According to the
invention, the *Agrobacterium tumefaciens* anthranilate synthase is particularly
useful for producing high levels of tryptophan.

To generate high levels of tryptophan in a plant or a selected host cell, the
selected anthranilate synthase nucleic acid is isolated and may be manipulated *in*
30 *vitro* to include regulatory signals required for gene expression in plant cells or
other cell types. Because the tryptophan biosynthetic pathway in plants is
reported to be present within plastids, the exogenous anthranilate synthase
nucleic acids are either introduced into plastids or are modified by adding a

nucleic acid segment encoding an amino-terminal plastid transit peptide. Such a plastid transit peptide can direct the anthranilate synthase gene product into plastids. In some instances the anthranilate synthase may already contain a plastid transport sequence, in which case there is no need to add one.

- 5 In order to alter the biosynthesis of tryptophan, the nucleic acid encoding an anthranilate synthase activity must be introduced into plant cells or other host cells and these transformed cells identified, either directly or indirectly. An entire anthranilate synthase or a useful portion or domain thereof can be used. The anthranilate synthase is stably incorporated into the plant cell genome. The transcriptional signals controlling expression of the anthranilate synthase must be
10 recognized by and be functional within the plant cells or other host cells. That is, the anthranilate synthase must be transcribed into messenger RNA, and the mRNA must be stable in the plant cell nucleus and be transported intact to the cytoplasm for translation. The anthranilate synthase mRNA must have
15 appropriate translational signals to be recognized and properly translated by plant cell ribosomes. The polypeptide gene product must substantially escape proteolytic attack in the cytoplasm, be transported into the correct cellular compartment (e.g. a plastid) and be able to assume a three-dimensional conformation that will confer enzymatic activity. The anthranilate synthase must
20 further be able to function in the biosynthesis of tryptophan and its derivatives; that is, it must be localized near the native plant enzymes catalyzing the flanking steps in biosynthesis (presumably in a plastid) in order to obtain the required substrates and to pass on the appropriate product.

- Even if all these conditions are met, successful overproduction of
25 tryptophan is not a predictable event. The expression of some transgenes may be negatively affected by nearby chromosomal elements. If the high level of tryptophan is achieved by mutation to reduce feedback inhibition, there may be other control mechanisms compensating for the reduced regulation at the anthranilate synthase step. There may be mechanisms that increase the rate of
30 breakdown of the accumulated amino acids. Tryptophan and related amino acids must be also overproduced at levels that are not toxic to the plant. Finally, the introduced trait must be stable and heritable in order to permit commercial development and use.

Isolation and Identification of DNA Coding for an Anthranilate Synthase

Nucleic acids encoding an anthranilate synthase can be identified and isolated by standard methods, for example, as described by Sambrook et al.,
 5 Molecular Cloning: A Laboratory Manual, 2nd Edition (1989); Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd Edition (January 15, 2001). For example, a DNA sequence encoding an anthranilate synthase or a domain thereof can be identified by screening of a DNA or cDNA library generated from nucleic acid derived from a particular cell type, cell line, primary
 10 cells, or tissue. Examples of libraries useful for identifying and isolating an anthranilate synthase include, but are not limited to, a cDNA library derived from *Agrobacterium tumefaciens* strain A348, maize inbred line B73 (Stratagene, La Jolla, California, Cat. #937005, Clontech, Palo Alto, California, Cat. # FL1032a, #FL1032b, and FL1032n), genomic library from maize inbred
 15 line Mo17 (Stratagene, Cat. #946102), genomic library from maize inbred line B73 (Clontech, Cat. # FL1032d), genomic DNA from *Anabaena* M22983 (e.g., Genbank Accession No. GI 152445), *Arabidopsis thaliana*, *Azospirillum brasilense* (e.g., Genbank Accession No. GI 1174156), *Brucella melitensis* (GI 17982357), *Escherichia coli*, *Euglena gracilis*, *Mesorhizobium loti* (e.g.,
 20 Genbank Accession No. GI 13472468), *Nostoc* sp. PCC7120 (e.g., Genbank Accession No. GI 17227910 or GI 17230725), *Rhizobium meliloti* (e.g., Genbank Accession No. GI 95177), *Ruta graveolens*, *Rhodopseudomonas palustris*, *Salmonella typhimurium*, *Serratia marcescens*, *Sulfolobus solfataricus*, soybean, rice, cotton, wheat, tobacco *Zea mays* (maize) or other species. Moreover,
 25 anthranilate synthase nucleic acids can be isolated by nucleic acid amplification procedures using genomic DNA, mRNA or cDNA isolated from any of these species.

Screening for DNA fragments that encode all or a portion of the sequence encoding an anthranilate synthase can be accomplished by screening plaques
 30 from a genomic or cDNA library for hybridization to a probe of an anthranilate synthase gene from other organisms or by screening plaques from a cDNA expression library for binding to antibodies that specifically recognize anthranilate synthase. DNA fragments that hybridize to anthranilate synthase

probes from other organisms and/or plaques carrying DNA fragments that are immunoreactive with antibodies to anthranilate synthase can be subcloned into a vector and sequenced and/or used as probes to identify other cDNA or genomic sequences encoding all or a portion of the desired anthranilate synthase gene.

- 5 Preferred cDNA probes for screening a maize or plant library can be obtained from plasmid clones pDPG600 or pDPG602.

A cDNA library can be prepared, for example, by random oligo priming or oligo dT priming. Plaques containing DNA fragments can be screened with probes or antibodies specific for anthranilate synthase. DNA fragments encoding
10 a portion of an anthranilate synthase gene can be subcloned and sequenced and used as probes to identify a genomic anthranilate synthase gene. DNA fragments encoding a portion of a bacterial or plant anthranilate synthase can be verified by determining sequence homology with other known anthranilate synthase genes or by hybridization to anthranilate synthase-specific messenger RNA. Once cDNA
15 fragments encoding portions of the 5', middle and 3' ends of an anthranilate synthase are obtained, they can be used as probes to identify and clone a complete genomic copy of the anthranilate synthase gene from a genomic library.

Portions of the genomic copy or copies of an anthranilate synthase gene can be sequenced and the 5' end of the gene identified by standard methods
20 including either by DNA sequence homology to other anthranilate synthase genes or by RNAase protection analysis, for example, as described by Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edition (1989); Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, 3rd Edition (January 15, 2001). The 3' and 5' ends of the target gene can also be located by computer
25 searches of genomic sequence databases using known AS coding regions. Once portions of the 5' end of the gene are identified, complete copies of the anthranilate synthase gene can be obtained by standard methods, including cloning or polymerase chain reaction (PCR) synthesis using oligonucleotide primers complementary to the DNA sequence at the 5' end of the gene. The
30 presence of an isolated full-length copy of the anthranilate synthase gene can be verified by hybridization, partial sequence analysis, or by expression of a maize anthranilate synthase enzyme.

Exemplary isolated DNAs of the invention include DNAs having the following nucleotide SEQ ID NO:

- 5 SEQ ID NO:1 -- *Agrobacterium tumefaciens* (wild type)
 SEQ ID NO:2 -- *Zea mays* (wild type)
 SEQ ID NO:3 -- *Ruta graveolens*
 SEQ ID NO:46 -- truncated TrpE gene of *E. coli* EMG2 (K-12 wt F+)
- 10 SEQ ID NO:67 -- *Zea mays* (C28 mutant)
 SEQ ID NO:68 -- *Zea mays* (C28 + terminator)
 SEQ ID NO:71 -- Chloroplast Targeting Peptide (g)
 SEQ ID NO:73 -- Chloroplast Targeting Peptide (a)
 SEQ ID NO:75 -- *Agrobacterium tumefaciens* (optimized)
 SEQ ID NO:76 -- *Rhodospseudomonas palustris*
 SEQ ID NO:83 -- *Rhodospseudomonas palustris* (RhoPa_TrpEG)
- 15 SEQ ID NO:84 -- *Agrobacterium tumefaciens* V48F mutant
 SEQ ID NO:85 -- *Agrobacterium tumefaciens* V48Y mutant
 SEQ ID NO:86 -- *Agrobacterium tumefaciens* S51F mutant
 SEQ ID NO:87 -- *Agrobacterium tumefaciens* S51C mutant
 SEQ ID NO:88 -- *Agrobacterium tumefaciens* N52F mutant
- 20 SEQ ID NO:89 -- *Agrobacterium tumefaciens* P293A mutant
 SEQ ID NO:90 -- *Agrobacterium tumefaciens* P293G mutant
 SEQ ID NO:91 -- *Agrobacterium tumefaciens* F298W mutant
 SEQ ID NO:92 -- *Agrobacterium tumefaciens* S50K mutant
 SEQ ID NO:93 -- *Agrobacterium tumefaciens* F298A mutant
- 25 SEQ ID NO:94 -- rice
 SEQ ID NO:95 -- rice isozyme
 SEQ ID NO:96 -- maize (U.S. Patent 6,118,047 to Anderson)
 SEQ ID NO:97 -- wheat
 SEQ ID NO:98 -- tobacco
- 30 Certain primers are also useful for the practise of the invention, for example, primers having SEQ ID NO:9-42, 47-56.

The invention also contemplates any isolated nucleic acid encoding an anthranilate synthase having, for example, any one of the following amino acid sequences.

- 5 SEQ ID NO:4 *Agrobacterium tumefaciens* (wild type)
- SEQ ID NO:5 *Zea mays* (wild type)
- SEQ ID NO:6 *Ruta graveolens*
- SEQ ID NO:7 *Rhizobium meliloti*
- SEQ ID NO:8 *Sulfolobus solfataricus*
- SEQ ID NO:43 *Rhizobium meliloti*
- 10 SEQ ID NO:44 *Sulfolobus solfataricus*
- SEQ ID NO:45 *Arabidopsis thaliana*
- SEQ ID NO:57 *Rhodopseudomonas palustris*
- SEQ ID NO:58 *Agrobacterium tumefaciens* V48F mutant
- SEQ ID NO:59 *Agrobacterium tumefaciens* V48Y mutant
- 15 SEQ ID NO:60 *Agrobacterium tumefaciens* S51F mutant
- SEQ ID NO:61 *Agrobacterium tumefaciens* S51C mutant
- SEQ ID NO:62 *Agrobacterium tumefaciens* N52F mutant
- SEQ ID NO:63 *Agrobacterium tumefaciens* P293A mutant
- SEQ ID NO:64 *Agrobacterium tumefaciens* P293G mutant
- 20 SEQ ID NO:65 *Agrobacterium tumefaciens* F298W mutant
- SEQ ID NO:66 *Zea mays* C28 mutant
- SEQ ID NO:69 *Agrobacterium tumefaciens* S50K mutant
- SEQ ID NO:70 *Agrobacterium tumefaciens* F298A mutant
- SEQ ID NO:74 Chloroplast Targeting Peptide (a)
- 25 SEQ ID NO:72 Chloroplast Targeting Peptide (g)
- SEQ ID NO:77 *Mesorhizobium loti* (MesLo_13472468)
- SEQ ID NO:78 *Azospirillum brasilense* (AzoBr_1717765)
- SEQ ID NO:79 *Brucella melitensis* (BruMe_17986732)
- SEQ ID NO:80 *Nostoc sp.* (Nostoc_17227910)
- 30 SEQ ID NO:81 *Nostoc sp.* (Nostoc_17230725)
- SEQ ID NO:82 *Rhodopseudomonas palustris* RhoPa_TrpEG
- SEQ ID NO:99 -- rice
- SEQ ID NO:100 -- rice isozyme

SEQ ID NO:101 -- maize (U.S. Patent 6,118,047 to Anderson)

SEQ ID NO:102 -- wheat

SEQ ID NO:103 -- tobacco

- Any of these nucleic acids and polypeptides can be utilized in the practice of the invention, as well as any mutant, variant or derivative thereof.

Monomeric Anthranilate Synthases

According to the invention, monomeric anthranilate synthases from plant and non-plant species are functional in plants and can provide high levels of tryptophan. Surprisingly, monomeric anthranilate synthases from non-plant species function very well in plants even though the sequences of these monomeric anthranilate synthases have low homology with most plant anthranilate synthases. For example, monomeric anthranilate synthases from species as diverse as bacteria, protists, and microbes can be used successfully. In particular, monomeric anthranilate synthases from bacterial species such as *Agrobacterium tumefaciens*, *Rhizobium meliloti*, *Mesorhizobium loti*, *Brucella melitensis*, *Nostoc sp.* PCC7120, *Azospirillum brasilense* and *Anabaena* M22983 are functional in plants and can provide high levels of tryptophan, despite the rather low sequence identity of these monomeric anthranilate synthases with most plant anthranilate synthases.

- Transgenic plants containing, for example, the wild type monomeric *Agrobacterium tumefaciens* anthranilate synthase can produce up to about 10,000 to about 12,000 ppm tryptophan in seeds, with average trp levels ranging up to about 7,000 to about 8,000 ppm. Non-transgenic soybean plants normally have up to only about 100 to about 200 ppm tryptophan in seeds. By comparison transgenic plants containing an added mutant *Zea mays* α domain produce somewhat lower levels of tryptophan (e.g., averages up to about 3000 to about 4000 ppm).

- Monomeric enzymes may have certain advantages over multimeric enzymes. For example, while the invention is not to be limited to a specific mechanism, a monomeric enzyme may provide greater stability, coordinated expression, and the like. When domains or subunits of a heterotetrameric anthranilate synthase are synthesized *in vivo*, those domains/subunits must properly assemble into a heterotetrameric form before the enzyme becomes active. Addition of a single domain of anthranilate synthase by transgenic means to a plant may not provide

overproduction of the entire heterotetrameric enzyme because there may not be sufficient endogenous amounts of the non-transgenic domains to substantially increase levels of the functional tetramer. Hence, nucleic acids, vectors and enzymes encoding a monomeric anthranilate synthase can advantageously be used to overproduce all of the enzymatic functions of anthranilate synthase.

According to the invention, anthranilate synthase domains from species that naturally produce heterotetrameric anthranilate synthases can be fused or linked to provide monomeric anthranilate synthases that can generate high tryptophan levels when expressed within a plant cell, plant tissue or seed. For example, a monomeric anthranilate synthase can be made by fusing or linking the α and β domains of anthranilate synthase so that the sequence of the α - β fusion generally aligns with an anthranilate synthase that is naturally monomeric. Examples of sequence alignments of monomeric and heterotetrameric anthranilate synthases are shown in Figures 21 and 35. Using such sequence alignments, the spacing and orientation of anthranilate synthase domains can be adjusted or modified to generate a monomeric anthranilate construct from heterotetrameric domains that optimally aligns with naturally monomeric anthranilate synthases. Such a fusion protein can be used to increase tryptophan levels in the tissues of a plant.

Heterotetrameric anthranilate synthases, such as the *Sulfolobus solfataricus* anthranilate synthase (e.g., Genbank Accession No. GII004323), share between about 30% to about 87% sequence homology with heterotetrameric anthranilate synthases from other plant and microbial species. Monomeric anthranilate synthases, such as the *A. tumefaciens* anthranilate synthase, have between about 83% and about 52% identity to the other monomeric enzymes such as *Rhizobium meliloti* (Genbank Accession No. GI 15966140) and *Azospirillum brasilense* (Genbank Accession No. 1717765), respectively. Bae et al., *Rhizobium meliloti* anthranilate synthase gene: cloning, sequence, and expression in *Escherichia coli*. *J. Bacteriol.* 171, 3471–3478 (1989); De Troch et al., Isolation and characterization of the *Azospirillum brasilense* trpE(G) gene, encoding anthranilate synthase. *Curr. Microbiol.* 34, 27–32 (1997).

However, the overall sequence identity shared between naturally monomeric and naturally heterotetrameric anthranilate synthases can be less than 30%. Hence, visual alignment rather than computer-generated alignment, may be needed to

optimally align monomeric and heterotetrameric anthranilate synthases. Landmark structures and sequences within the anthranilate synthases can facilitate sequences alignments. For example, the motif "LLES" is part of a β -sheet of the β -sandwich that forms the tryptophan-binding pocket of anthranilate synthases. Such landmark sequences can be used to more confidently align divergent anthranilate synthase sequences, and are especially useful for determination of key residues involved in tryptophan binding.

To accomplish the fusion or linkage of anthranilate synthase domains, the C-terminus of the selected TrpE or α -domain is linked to the N-terminus of the TrpG domain or β -domain. In some cases, a linker peptide may be utilized between the domains to provide the appropriate spacing and/or flexibility. Appropriate linker sequences can be identified by sequence alignment of monomeric and heterotetrameric anthranilate synthases.

The selected β -domains can be cloned, for example, by hybridization, PCR amplification or as described in Anderson et al., U.S. Pat. No. 6,118,047. A plastid transit peptide sequence can also be linked to the anthranilate synthase coding region using standard methods. For example, an *Arabidopsis* small subunit (SSU) chloroplast targeting peptide (CTP, SEQ ID NO:71-74) may be used for this purpose. See also, Stark et al., (1992) Science 258: 287. The fused gene can then be inserted into a suitable vector for plant transformation as described herein.

Anthranilate Synthase Mutants

Mutant anthranilate synthases contemplated by the invention can have any type of mutation including, for example, amino acid substitutions, deletions, insertions and/or rearrangements. Such mutants can be derivatives or variants of anthranilate synthase nucleic acids and polypeptides specifically identified herein. Alternatively, mutant anthranilate synthases can be obtained from any available species, including those not explicitly identified herein. The mutants, derivatives and variants can have identity with at least about 30% of the amino acid positions of any one of SEQ ID NO:4-8, 43-45, 57-66, 69-70, 77-82, 99-103 and have anthranilate synthase activity. In a preferred embodiment, polypeptide derivatives and variants have identity with at least about 50% of the amino acid

positions of any one of SEQ ID NO:4-8, 43-45, 57-66, 69-70, 77-82, 99-103 and have anthranilate synthase activity. In a more preferred embodiment, polypeptide derivatives and variants have identity with at least about 60% of the amino acid positions of any one of SEQ ID NO:4-8, 43-45, 57-66, 69-70, 77-82, 99-103 and have anthranilate synthase activity. In a more preferred embodiment, polypeptide derivatives and variants have identity with at least about 70% of the amino acid positions of any one of SEQ ID NO:4-8, 43-45, 57-66, 69-70, 77-82, 99-103 and have anthranilate synthase activity. In an even more preferred embodiment, polypeptide derivatives and variants have identity with at least about 80% of the amino acid positions of any one of SEQ ID NO:4-8, 43-45, 57-66, 69-70, 77-82, 99-103 and have anthranilate synthase activity. In an even more preferred embodiment, polypeptide derivatives and variants have identity with at least about 90% of the amino acid positions of any one of SEQ ID NO:4-8, 43-45, 57-66, 69-70, 77-82, 99-103 and have anthranilate synthase activity. In an even more preferred embodiment, polypeptide derivatives and variants have identity with at least about 95% of the amino acid positions of any one of SEQ ID NO:4-8, 43-45, 57-66, 69-70, 77-82, 99-103 and have anthranilate synthase activity.

In one embodiment, anthranilate synthase mutants, variants and derivatives can be identified by hybridization of any one of SEQ ID NO:1-3, 9-42, 46, 47-56, 67-68, 75-76, 83-98, or a fragment or primer thereof under moderate or, preferably, high stringency conditions to a selected source of nucleic acids. Moderate and stringent hybridization conditions are well known to the art, see, for example sections 0.47-9.51 of Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edition (1989); *see also*, Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd Edition (January 15, 2001). For example, stringent conditions are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate (SSC); 0.1% sodium lauryl sulfate (SDS) at 50°C, or (2) employ a denaturing agent such as formamide during hybridization, e.g., 50% formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is use of 50%

formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% sodium dodecylsulfate (SDS), and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1%

5 SDS.

The invention further provides hybridization probes and primers comprising a novel isolated and purified DNA segment of at least seven nucleotide bases, which can be detectably labeled or bind to a detectable label. Such a hybridization probe or primer can hybridize under moderate or high
10 stringency conditions to either strand of a DNA molecule that encodes an anthranilate synthase. Examples of such hybridization probes and primers include any one of SEQ ID NO:9-42, 47-56.

The anthranilate synthase can be any anthranilate synthase, or a mutant or domain thereof, such as the α -domain. The anthranilate synthase can be a
15 monomeric anthranilate synthase. Functional mutants are preferred, particularly those that can generate high levels of tryptophan in a plant, for example, those mutants that are substantially resistant to inhibition by an amino acid analog of tryptophan.

Nucleic acids encoding mutant anthranilate synthases can also be
20 generated from any convenient species, for example, from nucleic acids encoding any domain of *Agrobacterium tumefaciens*, *Anabaena* M22983 (e.g. Genbank Accession No. GI 152445), *Arabidopsis thaliana*, *Azospirillum brasilense* (e.g., Genbank Accession No. GI 1174156), *Brucella melitensis* (e.g., Genbank Accession No. GI 17982357), *Escherichia coli*, *Euglena gracilis*,
25 *Mesorhizobium loti* (e.g., Genbank Accession No. GI 13472468), *Nostoc* sp. PCC7120 (e.g., Genbank Accession No. GI 17227910 or GI 17230725), *Rhizobium meliloti* (e.g., Genbank Accession No. GI 95177), *Ruta graveolens*, *Rhodopseudomonas palustris*, *Salmonella typhimurium*, *Serratia marcescens*, *Sulfolobus solfataricus*, soybean, rice, cotton, wheat, tobacco *Zea mays* (maize)
30 or any gene encoding a subunit or domain of anthranilate synthase.

Mutants having increased anthranilate synthase activity, reduced sensitivity to feedback inhibition by tryptophan or analogs thereof, and/or the ability to generate increased amounts of tryptophan in a plant are desirable. Such

mutants do have a functional change in the level or type of activity they exhibit and are sometimes referred to as “derivatives” of the anthranilate synthase nucleic acids and polypeptides provided herein.

However, the invention also contemplates anthranilate synthase variants as well as anthranilate synthase nucleic acids with “silent” mutations. As used herein, a silent mutation is a mutation that changes the nucleotide sequence of the anthranilate synthase but that does not change the amino acid sequence of the encoded anthranilate synthase. A variant anthranilate synthase is encoded by a mutant nucleic acid and the variant has one or more amino acid changes that do not substantially change its activity when compared to the corresponding wild type anthranilate synthase. The invention is directed to all such derivatives, variants and anthranilate synthases nucleic acids with silent mutations.

DNA encoding a mutated anthranilate synthase that is resistant and/or tolerant to L-tryptophan or amino acid analogs of tryptophan can be obtained by several methods. The methods include, but are not limited to:

1. spontaneous variation and direct mutant selection in cultures;
2. direct or indirect mutagenesis procedures on tissue cultures of any cell types or tissue, seeds or plants;
3. mutation of the cloned anthranilate synthase gene by methods such as by chemical mutagenesis; site specific or site directed mutagenesis (Sambrook et al., cited *supra*), transposon mediated mutagenesis (Berg et al., Biotechnology, 1, 417 (1983)), and deletion mutagenesis (Mitra et al., Molec. Gen. Genetic., 215, 294 (1989));
4. rational design of mutations in key residues; and
5. DNA shuffling to incorporate mutations of interest into various anthranilate synthase nucleic acids.

For example, protein structural information from available anthranilate synthase proteins can be used to rationally design anthranilate synthase mutants that have a high probability of having increased activity or reduced sensitivity to tryptophan or tryptophan analogs. Such protein structural information is available, for example, on the *Solfulobus solfataricus* anthranilate synthase (Knochel et. al., Proc. Natl. Acad. Sci. USA, 96, 9479-9484 (1999)). Rational design of mutations can be accomplished by alignment of the selected

anthranilate synthase amino acid sequence with the anthranilate synthase amino acid sequence from an anthranilate synthase of known structure, for example, *Sulfolobus solfataricus*. See Figures 6, 21 and 35. The predicted tryptophan binding and catalysis regions of the anthranilate synthase protein can be assigned
5 by combining the knowledge of the structural information with the sequence homology. For example, residues in the tryptophan binding pocket can be identified as potential candidates for mutation to alter the resistance of the enzyme to feedback inhibition by tryptophan. Using such structural information, several *Agrobacterium tumefaciens* anthranilate synthase mutants were rationally
10 designed in the site or domain involved in tryptophan binding.

Using such sequence and structural analysis, regions analogous to the monomeric *Agrobacterium tumefaciens* anthranilate synthase at approximately positions 25-60 or 200-225 or 290-300 or 370-375 were identified in the monomeric *Agrobacterium tumefaciens* anthranilate synthase as being
15 potentially useful residues for mutation to produce active anthranilate synthases that may have less sensitivity to tryptophan feedback inhibition. More specifically, amino acids analogous to P29, E30, S31, I32, S42, V43, V48, S50, S51, N52, N204, P205, M209, F210, G221, N292, P293, F298 and A373 in the monomeric *Agrobacterium tumefaciens* anthranilate synthase are being
20 potentially useful residues for mutation to produce active anthranilate synthases that may have less sensitivity to tryptophan feedback inhibition. The invention contemplates any amino acid substitution or insertion at any of these positions. Alternatively, the amino acid at any of these positions can be deleted.

Site directed mutagenesis can be used to generate amino acid
25 substitutions, deletions and insertions at a variety of sites. Examples of specific mutations made within the *Agrobacterium tumefaciens* anthranilate synthase coding region include the following:

- at about position 48 replace Val with Phe (see e.g., SEQ ID NO:58);
- at about position 48 replace Val with Tyr (see e.g., SEQ ID NO:59);
- 30 at about position 51 replace Ser with Phe (see e.g., SEQ ID NO:60);
- at about position 51 replace Ser with Cys (see e.g., SEQ ID NO:61);
- at about position 52 replace Asn with Phe (see e.g., SEQ ID NO:62);
- at about position 293 replace Pro with Ala (see e.g., SEQ ID NO:63);

at about position 293 replace Pro with Gly (see e.g., SEQ ID NO:64); or
at about position 298 replace Phe with Trp (see e.g., SEQ ID NO:65).

Similar mutations can be made in analogous positions of any anthranilate
synthase by alignment of the amino acid sequence of the anthranilate synthase to
5 be mutated with an *Agrobacterium tumefaciens* anthranilate synthase amino acid
sequence. One example of an *Agrobacterium tumefaciens* anthranilate synthase
amino acid sequence that can be used for alignment is SEQ ID NO:4.

Useful mutants can also be identified by classical mutagenesis and
genetic selection. A functional change can be detected in the activity of the
10 enzyme encoded by the gene by exposing the enzyme to free L-tryptophan or
amino acid analogs of tryptophan, or by detecting a change in the DNA molecule
using restriction enzyme mapping or DNA sequence analysis.

For example, a gene encoding an anthranilate synthase substantially
tolerant to 5-methyltryptophan can be isolated from a 5-methyltryptophan
15 tolerant cell line. See U.S. Patent No. 4,581,847, issued April 15, 1986, the
disclosure of which is incorporated by reference herein. Briefly, partially
differentiated plant cell cultures are grown and subcultured with continuous
exposures to low levels of 5-methyltryptophan. 5-methyltryptophan
concentrations are then gradually increased over several subculture intervals.
20 Cells or tissues growing in the presence of normally toxic 5-methyltryptophan
levels are repeatedly subcultured in the presence of 5-methyltryptophan and
characterized. Stability of the 5-methyltryptophan tolerance trait of the cultured
cells may be evaluated by growing the selected cell lines in the absence of 5-
methyltryptophan for various periods of time and then analyzing growth after
25 exposing the tissue to 5-methyltryptophan. Cell lines that are tolerant by virtue
of having an altered anthranilate synthase enzyme can be selected by identifying
cell lines having enzyme activity in the presence of normally toxic, i.e., growth
inhibitor, levels of 5-methyltryptophan.

The anthranilate synthase gene cloned from a 5-MT- or 6-MA-resistant
30 cell line can be assessed for tolerance to 5-MT, 6-MA, or other amino acid
analogues of tryptophan by standard methods, as described in U.S. Patent No.
4,581,847, issued April 15, 1986, the disclosure of which is incorporated by
reference herein.

Cell lines with an anthranilate synthase of reduced sensitivity to 5-methyltryptophan inhibition can be used to isolate a 5-methyltryptophan-resistant anthranilate synthase. A DNA library from a cell line tolerant to 5-methyltryptophan can be generated and DNA fragments encoding all or a portion of an anthranilate synthase gene can be identified by hybridization to a cDNA probe encoding a portion of an anthranilate synthase gene. A complete copy of the altered gene can be obtained either by cloning and ligation or by PCR synthesis using appropriate primers. The isolation of the altered gene coding for anthranilate synthase can be confirmed in transformed plant cells by determining whether the anthranilate synthase being expressed retains enzyme activity when exposed to normally toxic levels of 5-methyltryptophan. See, Anderson et al., U.S. Pat. No. 6,118,047.

Coding regions of any DNA molecule provided herein can also be optimized for expression in a selected organism, for example, a selected plant or other host cell type. An example of a DNA molecule having optimized codon usage for a selected plant is an *Agrobacterium tumefaciens* anthranilate synthase DNA molecule having SEQ ID NO:75. This optimized *Agrobacterium tumefaciens* anthranilate synthase DNA (SEQ ID NO:75) has 94% identity with SEQ ID NO:1.

20

Transgenes and Vectors

Once a nucleic acid encoding anthranilate synthase or a domain thereof is obtained and amplified, it is operably combined with a promoter and, optionally, with other elements to form a transgene.

Most genes have regions of DNA sequence that are known as promoters and which regulate gene expression. Promoter regions are typically found in the flanking DNA sequence upstream from the coding sequence in both prokaryotic and eukaryotic cells. A promoter sequence provides for regulation of transcription of the downstream gene sequence and typically includes from about 50 to about 2,000 nucleotide base pairs. Promoter sequences also contain regulatory sequences such as enhancer sequences that can influence the level of gene expression. Some isolated promoter sequences can provide for gene expression of heterologous genes, that is, a gene different from the native or

homologous gene. Promoter sequences are also known to be strong or weak or inducible. A strong promoter provides for a high level of gene expression, whereas a weak promoter provides for a very low level of gene expression. An inducible promoter is a promoter that provides for turning on and off of gene
5 expression in response to an exogenously added agent or to an environmental or developmental stimulus. Promoters can also provide for tissue specific or developmental regulation. An isolated promoter sequence that is a strong promoter for heterologous genes is advantageous because it provides for a sufficient level of gene expression to allow for easy detection and selection of
10 transformed cells and provides for a high level of gene expression when desired.

The promoter in a transgene of the invention can provide for expression of anthranilate synthase from a DNA sequence encoding anthranilate synthase. Preferably, the coding sequence is expressed so as to result in an increase in tryptophan levels within plant tissues, for example, within the seeds of the plant.
15 In another embodiment, the coding sequence is expressed so as to result in increased tolerance of the plant cells to feedback inhibition or to growth inhibition by an amino acid analog of tryptophan or so as to result in an increase in the total tryptophan content of the cells. The promoter can also be inducible so that gene expression can be turned on or off by an exogenously added agent.
20 For example, a bacterial promoter such as the P_{lac} promoter can be induced to varying levels of gene expression depending on the level of isothioproprylgalactoside added to the transformed bacterial cells. It may also be preferable to combine the gene with a promoter that provides tissue specific expression or developmentally regulated gene expression in plants. Many
25 promoters useful in the practice of the invention are available to those of skill in the art.

Preferred promoters will generally include, but are not limited to, promoters that function in bacteria, bacteriophage, plastids or plant cells. Useful promoters include the CaMV 35S promoter (Odell et al., Nature, 313, 810
30 (1985)), the CaMV 19S (Lawton et al., Plant Mol. Biol., 9, 31F (1987)), *nos* (Ebert et al., PNAS USA, 84, 5745 (1987)), *Adh* (Walker et al., PNAS USA, 84, 6624 (1987)), sucrose synthase (Yang et al., PNAS USA, 87, 4144 (1990)), α -tubulin, napin, actin (Wang et al., Mol. Cell. Biol., 12, 3399 (1992)), *cab*

(Sullivan et al., Mol. Gen. Genet., 215, 431 (1989)), PEPCase promoter (Hudspeth et al., Plant Mol. Biol., 12, 579 (1989)), the 7S-alpha'-conglycinin promoter (Beachy et al., EMBO J., 4, 3047 (1985)) or those associated with the R gene complex (Chandler et al., The Plant Cell, 1, 1175 (1989)). Other useful
5 promoters include the bacteriophage SP6, T3, and T7 promoters.

Plastid promoters can be also be used. Most plastid genes contain a promoter for the multi-subunit plastid-encoded RNA polymerase (PEP) as well as the single-subunit nuclear-encoded RNA polymerase. A consensus sequence for the nuclear-encoded polymerase (NEP) promoters and listing of specific
10 promoter sequences for several native plastid genes can be found in Hajdukiewicz et al., 1997, EMBO J. Vol. 16 pp. 4041-4048, which is hereby in its entirety incorporated by reference.

Examples of plastid promoters that can be used include the *Zea mays* plastid RRN (ZMRRN) promoter. The ZMRRN promoter can drive expression
15 of a gene when the *Arabidopsis thaliana* plastid RNA polymerase is present. Similar promoters that can be used in the present invention are the Glycine max plastid RRN (SOYRRN) and the Nicotiana tabacum plastid RRN (NTRRN) promoters. All three promoters can be recognized by the *Arabidopsis* plastid RNA polymerase. The general features of RRN promoters are described by
20 Hajdukiewicz et al. and U.S. Patent 6,218,145.

Moreover, transcription enhancers or duplications of enhancers can be used to increase expression from a particular promoter. Examples of such enhancers include, but are not limited to, elements from the CaMV 35S promoter and octopine synthase genes (Last et al., U.S. Patent No. 5,290,924, issued
25 March 1, 1994). For example, it is contemplated that vectors for use in accordance with the present invention may be constructed to include the *ocs* enhancer element. This element was first identified as a 16 bp palindromic enhancer from the octopine synthase (*ocs*) gene of *Agrobacterium* (Ellis et al., EMBO J., 6, 3203 (1987)), and is present in at least 10 other promoters (Bouchez
30 et al., EMBO J., 8, 4197 (1989)). It is proposed that the use of an enhancer element, such as the *ocs* element and particularly multiple copies of the element, will act to increase the level of transcription from adjacent promoters when applied in the context of monocot transformation. Tissue-specific promoters,

including but not limited to, root-cell promoters (Conkling et al., Plant Physiol., 93, 1203 (1990)), and tissue-specific enhancers (Fromm et al., The Plant Cell, 1, 977 (1989)) are also contemplated to be particularly useful, as are inducible promoters such as ABA- and turgor-inducible promoters, and the like.

5 As the DNA sequence between the transcription initiation site and the start of the coding sequence, i.e., the untranslated leader sequence, can influence gene expression, one may also wish to employ a particular leader sequence. Any leader sequence available to one of skill in the art may be employed. Preferred leader sequences direct optimum levels of expression of the attached gene, for
10 example, by increasing or maintaining mRNA stability and/or by preventing inappropriate initiation of translation (Joshi, Nucl. Acid Res., 15, 6643 (1987)). The choice of such sequences is at the discretion of those of skill in the art. Sequences that are derived from genes that are highly expressed in dicots, and in soybean in particular, are contemplated.

15 In some cases, extremely high expression of anthranilate synthase or a domain thereof, is not necessary. For example, using the methods of the invention such high levels of anthranilate synthase may be generated that the availability of substrate, rather than enzyme, may limit the levels of tryptophan generated. In such cases, more moderate or regulated levels of expression can be
20 selected by one of skill in the art. Such a skilled artisan can readily modulate or regulate the levels of expression, for example, by use of a weaker promoter or by use of a developmentally regulated or tissue specific promoter.

 Nucleic acids encoding the anthranilate synthase of interest can also include a plastid transit peptide (e.g. SEQ ID NO:72 or 74) to facilitate transport
25 of the anthranilate synthase polypeptide into plastids, for example, into chloroplasts. A nucleic acid encoding the selected plastid transit peptide (e.g. SEQ ID NO: 71 or 73) is generally linked in-frame with the coding sequence of the anthranilate synthase. However, the plastid transit peptide can be placed at either the N-terminal or C-terminal end of the anthranilate synthase.

30 Constructs also include the nucleic acid of interest (e.g. DNA encoding an anthranilate synthase) along with a nucleic acid sequence that acts as a transcription termination signal and that allows for the polyadenylation of the resultant mRNA. Such transcription termination signals are placed 3' or

downstream of the coding region of interest. Preferred transcription termination signals contemplated include the transcription termination signal from the nopaline synthase gene of *Agrobacterium tumefaciens* (Bevan et al., Nucl. Acid Res., 11, 369 (1983)), the terminator from the octopine synthase gene of *Agrobacterium tumefaciens*, and the 3' end of genes encoding protease inhibitor I or II from potato or tomato, although other transcription termination signals known to those of skill in the art are also contemplated. Regulatory elements such as Adh intron 1 (Callis et al., Genes Develop., 1, 1183 (1987)), sucrose synthase intron (Vasil et al., Plant Physiol., 91, 5175 (1989)) or TMV omega element (Gallie et al., The Plant Cell, 1, 301 (1989)) may further be included where desired. These 3' nontranslated regulatory sequences can be obtained as described in An, Methods in Enzymology, 153, 292 (1987) or are already present in plasmids available from commercial sources such as Clontech, Palo Alto, California. The 3' nontranslated regulatory sequences can be operably linked to the 3' terminus of an anthranilate synthase gene by standard methods. Other such regulatory elements useful in the practice of the invention are known to those of skill in the art.

Selectable marker genes or reporter genes are also useful in the present invention. Such genes can impart a distinct phenotype to cells expressing the marker gene and thus allow such transformed cells to be distinguished from cells that do not have the marker. Selectable marker genes confer a trait that one can 'select' for by chemical means, i.e., through the use of a selective agent (e.g., a herbicide, antibiotic, or the like). Reporter genes, or screenable genes, confer a trait that one can identify through observation or testing, i.e., by 'screening' (e.g., the R-locus trait). Of course, many examples of suitable marker genes are known to the art and can be employed in the practice of the invention.

Possible selectable markers for use in connection with the present invention include, but are not limited to, a *neo* gene (Potrykus et al., Mol. Gen. Genet., 199, 183 (1985)) which codes for neomycin resistance and can be selected for using kanamycin, G418, and the like; a *bar* gene which codes for bialaphos resistance; a gene which encodes an altered EPSP synthase protein (Hinchee et al., Biotech., 6, 915 (1988)) thus conferring glyphosate resistance; a nitrilase gene such as *bxn* from *Klebsiella ozaenae* which confers resistance to

bromoxynil (Stalker et al., Science, 242, 419 (1988)); a mutant acetolactate synthase gene (ALS) that confers resistance to imidazolinone, sulfonylurea or other ALS-inhibiting chemicals (European Patent Application 154,204, 1985); a methotrexate-resistant DHFR gene (Thillet et al., J. Biol. Chem., 263, 12500 (1988)); a dalapon dehalogenase gene that confers resistance to the herbicide dalapon; or a mutated anthranilate synthase gene that confers resistance to 5-methyl tryptophan. Where a mutant EPSP synthase gene is employed, additional benefit may be realized through the incorporation of a suitable plastid transit peptide (CTP).

10 An illustrative embodiment of a selectable marker gene capable of being used in systems to select transformants is the genes that encode the enzyme phosphinothricin acetyltransferase, such as the *bar* gene from *Streptomyces hygroscopicus* or the *pat* gene from *Streptomyces viridochromogenes* (U.S. Pat. No. 5,550,318, which is incorporated by reference herein). The enzyme
15 phosphinothricin acetyl transferase (PAT) inactivates the active ingredient in the herbicide bialaphos, phosphinothricin (PPT). PPT inhibits glutamine synthetase, (Murakami et al., Mol. Gen. Genet., 205, 42 (1986); Twell et al., Plant Physiol., 91, 1270 (1989)) causing rapid accumulation of ammonia and cell death.

Screenable markers that may be employed include, but are not limited to,
20 a β -glucuronidase or *uidA* gene (GUS) which encodes an enzyme for which various chromogenic substrates are known; an R-locus gene, which encodes a product that regulates the production of anthocyanin pigments (red color) in plant tissues (Dellaporta et al., in Chromosome Structure and Function, pp. 263-282 (1988)); a β -lactamase gene (Sutcliffe, PNAS USA, 75, 3737 (1978)), which
25 encodes an enzyme for which various chromogenic substrates are known (e.g., PADAC, a chromogenic cephalosporin); a *xyIE* gene (Zukowsky et al., PNAS USA, 80, 1101 (1983)) that encodes a catechol dioxygenase that can convert chromogenic catechols; an α -amylase gene (Ikuta et al., Biotech., 8, 241 (1990)); a tyrosinase gene (Katz et al., J. Gen. Microbiol., 129, 2703 (1983)) that encodes
30 an enzyme capable of oxidizing tyrosine to DOPA and dopaquinone which in turn condenses to form the easily detectable compound melanin; a β -galactosidase gene, which encodes an enzyme for which there are chromogenic substrates; a luciferase (*lux*) gene (Ow et al., Science, 234, 856 (1986)), which

allows for bioluminescence detection; or even an aequorin gene (Prasher et al., Biochem. Biophys. Res. Comm., 126, 1259 (1985)), which may be employed in calcium-sensitive bioluminescence detection, or a green fluorescent protein gene (Niedz et al., Plant Cell Reports, 14, 403 (1995)). The presence of the *lux* gene
5 in transformed cells may be detected using, for example, X-ray film, scintillation counting, fluorescent spectrophotometry, low-light video cameras, photon-counting cameras, or multiwell luminometry. It is also envisioned that this system may be developed for populational screening for bioluminescence, such as on tissue culture plates, or even for whole plant screening.

10 Additionally, transgenes may be constructed and employed to provide targeting of the gene product to an intracellular compartment within plant cells or in directing a protein to the extracellular environment. This will generally be achieved by joining a DNA sequence encoding a transit or signal peptide
15 sequence to the coding sequence of a particular gene. The resultant transit, or signal, peptide will transport the protein to a particular intracellular, or extracellular destination, respectively, and may then be post-translationally removed. Transit or signal peptides act by facilitating the transport of proteins through intracellular membranes, e.g., vacuole, vesicle, plastid and mitochondrial membranes, whereas signal peptides direct proteins through the
20 extracellular membrane. By facilitating transport of the protein into compartments inside or outside the cell, these sequences may increase the accumulation of gene product.

A particular example of such a use concerns the direction of an anthranilate synthase to a particular organelle, such as the plastid, rather than to
25 the cytoplasm. This is exemplified by the use of the *Arabidopsis* SSU1A transit peptide that confers plastid-specific targeting of proteins. Alternatively, the transgene can comprise a plastid transit peptide-encoding DNA sequence or a DNA sequence encoding the the *rbcS* (RuBISCO) transit peptide operably linked between a promoter and the DNA sequence encoding an anthranilate synthase
30 (for a review of plastid targeting peptides, see Heijne et al., Eur. J. Biochem., 180, 535 (1989); Keegstra et al., Ann. Rev. Plant Physiol. Plant Mol. Biol., 40, 471 (1989)). If the transgene is to be introduced into a plant cell, the transgene can also contain plant transcriptional termination and polyadenylation signals

and translational signals linked to the 3' terminus of a plant anthranilate synthase gene.

An exogenous plastid transit peptide can be used which is not encoded within a native plant anthranilate synthase gene. A plastid transit peptide is typically 40 to 70 amino acids in length and functions post-translationally to direct a protein to the plastid. The transit peptide is cleaved either during or just after import into the plastid to yield the mature protein. The complete copy of a gene encoding a plant anthranilate synthase may contain a plastid transit peptide sequence. In that case, it may not be necessary to combine an exogenously obtained plastid transit peptide sequence into the transgene.

Exogenous plastid transit peptide encoding sequences can be obtained from a variety of plant nuclear genes, so long as the products of the genes are expressed as preproteins comprising an amino terminal transit peptide and transported into plastid. Examples of plant gene products known to include such transit peptide sequences include, but are not limited to, the small subunit of ribulose biphosphate carboxylase, chlorophyll a/b binding protein, plastid ribosomal proteins encoded by nuclear genes, certain heatshock proteins, amino acid biosynthetic enzymes such as acetolactate acid synthase, 3-enolpyruvylphosphoshikimate synthase, dihydrodipicolinate synthase, anthranilate synthase and the like. In some instances a plastid transport protein already may be encoded in the anthranilate synthase gene of interest, in which case there may be no need to add such plastid transit sequences. Alternatively, the DNA fragment coding for the transit peptide may be chemically synthesized either wholly or in part from the known sequences of transit peptides such as those listed above.

Regardless of the source of the DNA fragment coding for the transit peptide, it should include a translation initiation codon, for example, an ATG codon, and be expressed as an amino acid sequence that is recognized by and will function properly in plastids of the host plant. Attention should also be given to the amino acid sequence at the junction between the transit peptide and the anthranilate synthase enzyme where it is cleaved to yield the mature enzyme. Certain conserved amino acid sequences have been identified and may serve as a guideline. Precise fusion of the transit peptide coding sequence with the

anthranilate synthase coding sequence may require manipulation of one or both DNA sequences to introduce, for example, a convenient restriction site. This may be accomplished by methods including site-directed mutagenesis, insertion of chemically synthesized oligonucleotide linkers, and the like.

5 Precise fusion of the nucleic acids encoding the plastid transport protein may not be necessary so long as the coding sequence of the plastid transport protein is in-frame with that of the anthranilate synthase. For example, additional peptidyl or amino acids can often be included without adversely affecting the expression or localization of the protein of interest.

10 Once obtained, the plastid transit peptide sequence can be appropriately linked to the promoter and an anthranilate synthase coding region in a transgene using standard methods. A plasmid containing a promoter functional in plant cells and having multiple cloning sites downstream can be constructed or obtained from commercial sources. The plastid transit peptide sequence can be
15 inserted downstream from the promoter using restriction enzymes. An anthranilate synthase coding region can then be translationally fused or inserted immediately downstream from and in frame with the 3' terminus of the plastid transit peptide sequence. Hence, the plastid transit peptide is preferably linked to the amino terminus of the anthranilate synthase. Once formed, the transgene can
20 be subcloned into other plasmids or vectors.

 In addition to nuclear plant transformation, the present invention also extends to direct transformation of the plastid genome of plants. Hence, targeting of the gene product to an intracellular compartment within plant cells may also be achieved by direct delivery of a gene to the intracellular compartment. Direct
25 transformation of plastid genome may provide additional benefits over nuclear transformation. For example, direct plastid transformation of anthranilate synthase eliminates the requirement for a plastid targeting peptide and post-translational transport and processing of the pre-protein derived from the corresponding nuclear transformants. Plastid transformation of plants has been
30 described by P. Maliga. *Current Opinion in Plant Biology* 5, 164-172 (2002), P. B. Heifetz. *Biochimie* vol. 82, 655-666 (2000), R.Bock. *J. Mol. Biol.* 312, 425-438 (2001), and H. Daniell et al., *Trends in Plant Science* 7, 84-91 (2002) and references within.

After constructing a transgene containing an anthranilate synthase gene, the cassette can then be introduced into a plant cell. Depending on the type of plant cell, the level of gene expression, and the activity of the enzyme encoded by the gene, introduction of DNA encoding an anthranilate synthase into the plant cell can lead to the overproduction of tryptophan, confer tolerance to an amino acid analog of tryptophan, such as 5-methyltryptophan or 6-methylantranilate, and/or otherwise alter the tryptophan content of the plant cell.

10 Transformation of Host Cells

A transgene comprising an anthranilate synthase gene can be subcloned into a known expression vector, and AS expression can be detected and/or quantitated. This method of screening is useful to identify transgenes providing for an expression of an anthranilate synthase gene, and expression of an anthranilate synthase in the plastid of a transformed plant cell.

Plasmid vectors include additional DNA sequences that provide for easy selection, amplification, and transformation of the transgene in prokaryotic and eukaryotic cells, e.g., pUC-derived vectors, pSK-derived vectors, pGEM-derived vectors, pSP-derived vectors, or pBS-derived vectors. The additional DNA sequences include origins of replication to provide for autonomous replication of the vector, selectable marker genes, preferably encoding antibiotic or herbicide resistance, unique multiple cloning sites providing for multiple sites to insert DNA sequences or genes encoded in the transgene, and sequences that enhance transformation of prokaryotic and eukaryotic cells.

Another vector that is useful for expression in both plant and prokaryotic cells is the binary Ti plasmid (as disclosed in Schilperoort et al., U.S. Patent No. 4,940,838, issued July 10, 1990) as exemplified by vector pGA582. This binary Ti plasmid vector has been previously characterized by An, cited *supra*. This binary Ti vector can be replicated in prokaryotic bacteria such as *E. coli* and *Agrobacterium*. The *Agrobacterium* plasmid vectors can also be used to transfer the transgene to plant cells. The binary Ti vectors preferably include the nopaline T DNA right and left borders to provide for efficient plant cell transformation, a selectable marker gene, unique multiple cloning sites in the T

border regions, the *colE1* replication of origin and a wide host range replicon. The binary Ti vectors carrying a transgene of the invention can be used to transform both prokaryotic and eukaryotic cells, but is preferably used to transform plant cells. See, for example, Glassman et al., U.S. Pat. No.

5 5,258,300.

The expression vector can then be introduced into prokaryotic or eukaryotic cells by available methods. Methods of transformation especially effective for monocots and dicots, include, but are not limited to, microprojectile bombardment of immature embryos (U.S. Pat. No. 5,990,390) or Type II
10 embryogenic callus cells as described by W.J. Gordon-Kamm et al. (Plant Cell, 2, 603 (1990)), M.E. Fromm et al. (Bio/Technology, 8, 833 (1990)) and D.A. Walters et al. (Plant Molecular Biology, 18, 189 (1992)), or by electroporation of type I embryogenic calluses described by D'Halluin et al. (The Plant Cell, 4, 1495 (1992)), or by Krzyzek (U.S. Patent No. 5,384,253, issued January 24,
15 1995). Transformation of plant cells by vortexing with DNA-coated tungsten whiskers (Coffec et al., U.S. Patent No. 5,302,523, issued April 12, 1994) and transformation by exposure of cells to DNA-containing liposomes can also be used.

After transformation of the selected anthranilate synthase construct into a
20 host cell, the host cell may be used for production of useful products generated by the transgenic anthranilate synthase in combination with the host cell's enzymatic machinery. Culturing the transformed cells can lead to enhanced production of tryptophan and other useful compounds, which can be recovered from the cells or from the culture media. Examples of useful compounds that
25 may be generated upon expression in a variety of host cells and/or organisms include tryptophan, indole acetic acid and other auxins, isoflavonoid compounds important to cardiovascular health found in soy, volatile indole compounds which act as signals to natural enemies of herbivorous insects in maize, anticarcinogens such as indole glucosinolates (indole-3-carbinol) found in the
30 Cruciferae plant family, as well as indole alkaloids such as ergot compounds produced by certain species of fungi. (Barnes et al., Adv Exp Med Biol, 401, 87 (1996); Frey et al., Proc Natl Acad Sci, 97, 14801 (2000); Muller et al., Biol Chem, 381, 679 (2000); Mantegani et al., Farmaco, 54, 288 (1999); Zeligs, J

Med Food, 1, 67 (1998); Mash et al., Ann NY Acad Sci, 844, 274 (1998); Melanson et al., Proc Natl Acad Sci, 94, 13345 (1997); Broadbent et al., Curr Med Chem, 5, 469 (1998)).

- Accumulation of tryptophan may also lead to the increased production of secondary metabolites in microbes and plants, for example, indole containing metabolites such as simple indoles, indole conjugates, indole alkaloids, indole phytoalexins and indole glucosinates in plants.

- Anthranilate synthases insensitive to tryptophan have the potential to increase a variety of chorismate-derived metabolites, including those derived from phenylalanine due to the stimulation of phenylalanine synthesis by tryptophan via chorismate mutase. See Siehl, D. The biosynthesis of tryptophan, tyrosine, and phenylalanine from chorismate in *Plant Amino Acids: Biochemistry and Biotechnology*, ed. BK Singh, pp 171-204. Other chorismate-derived metabolites that may increase when feedback insensitive anthranilate synthases are present include phenylpropanoids, flavonoids, and isoflavonoids, as well as those derived from anthranilate, such as indole, indole alkaloids, and indole glucosinolates. Many of these compounds are important plant hormones, plant defense compounds, chemopreventive agents of various health conditions, and/or pharmacologically active compounds.

- The range of these compounds whose synthesis might be increased by expression of anthranilate synthase depends on the organism in which the anthranilate synthase is expressed. One of skill in the art can readily assess which organisms and host cells to use and/or test in order to generate the desired compounds. The invention contemplates synthesis of tryptophan and other useful compounds in a variety of organisms, including plants, microbes, fungi, yeast, bacteria, insect cells, and mammalian cells.

Strategy for Selection of Tryptophan Overproducer Cell Lines

- Efficient selection of a desired tryptophan analog resistant, tryptophan overproducer variant using tissue culture techniques requires careful determination of selection conditions. These conditions are optimized to allow growth and accumulation of tryptophan analog resistant, tryptophan overproducer cells in the culture while inhibiting the growth of the bulk of the

cell population. The situation is complicated by the fact that the vitality of individual cells in a population can be highly dependent on the vitality of neighboring cells.

Conditions under which cell cultures are exposed to tryptophan analog are determined by the characteristics of the interaction of the compound with the tissue. Such factors as the degree of toxicity and the rate of inhibition should be considered. The accumulation of the compounds by cells in culture, and the persistence and stability of the compounds, both in the media and in the cells, also need to be considered along with the extent of uptake and transmission to the desired cellular compartment. Additionally, it is important to determine whether the effects of the compounds can be readily reversed by the addition of tryptophan.

The effects of the analog on culture viability and morphology is carefully evaluated. It is especially important to choose analog exposure conditions that have no impact on plant regeneration capability of cultures. Choice of analog exposure conditions is also influenced by whether the analog kills cells or simply inhibits cell divisions.

The choice of a selection protocol is dependent upon the considerations described above. The protocols briefly described below can be utilized in the selection procedure. For example, to select for cells that are resistant to growth inhibition by a tryptophan analog, finely divided cells in liquid suspension culture can be exposed to high tryptophan analog levels for brief periods of time. Surviving cells are then allowed to recover and accumulate and are then reexposed for subsequently longer periods of time. Alternatively, organized partially differentiated cell cultures are grown and subcultured with continuous exposure to initially low levels of a tryptophan analog. Concentrations are then gradually increased over several subculture intervals. While these protocols can be utilized in a selection procedure, the present invention is not limited to these procedures.

Genes for Plant Modification

As described hereinabove, genes that function as selectable marker genes and reporter genes can be operably combined with the DNA sequence encoding

the anthranilate synthase, or domain thereof, in transgenes, vectors and plants of the present invention. Additionally, other agronomical traits can be added to the transgenes, vectors and plants of the present invention. Such traits include, but are not limited to, insect resistance or tolerance; disease resistance or tolerance
5 (viral, bacterial, fungal, nematode); stress resistance or tolerance, as exemplified by resistance or tolerance to drought, heat, chilling, freezing, excessive moisture, salt stress, oxidative stress; increased yields; food content and makeup; physical appearance; male sterility; drydown; standability; prolificacy; starch properties; oil quantity and quality; and the like. One may incorporate one or more genes
10 conferring such traits into the plants of the invention.

Insect Resistance or Tolerance

Bacillus thuringiensis (or "Bt") bacteria include nearly 20 known subspecies of bacteria which produce endotoxin polypeptides that are toxic when ingested by a wide variety of insect species. The biology and molecular biology
15 of the endotoxin proteins (Bt proteins) and corresponding genes (Bt genes) has been reviewed by H. R. Whitely et al., Ann. Rev. Microbiol., **40**, 549 (1986) and by H. Hofte et al., Microbiol. Rev., **53**, 242 (1989). Genes coding for a variety of Bt proteins have been cloned and sequenced. A segment of the Bt polypeptide is essential for toxicity to a variety of *Lepidoptera* pests and is contained within
20 approximately the first 50% of the Bt polypeptide molecule. Consequently, a truncated Bt polypeptide coded by a truncated Bt gene will in many cases retain its toxicity towards a number of *Lepidoptera* insect pests. For example, the HD73 and HD1 Bt polypeptides have been shown to be toxic to the larvae of the important *Lepidoptera* insect pests of plants in the USA such as the European
25 corn borer, cutworms and earworms. The genes coding for the HD1 and HD73 Bt polypeptides have been cloned and sequenced by M. Geiser et al., Gene, **48**, 109 (1986) and M. J. Adang et al., Gene, **36**, 289 (1985), respectively, and can be cloned from HD1 and HD73 strains obtained from culture collections (e.g. Bacillus Genetic Stock Center, Columbus, Ohio or USDA Bt stock collection
30 Peoria, Ill.) using standard protocols. Examples of Bt genes and polypeptides are described, for example, in U.S. Patent Numbers 6,329,574, 6,303,364, 6,320,100 and 6,331,655.

DNA coding for new, previously uncharacterized Bt toxins, may be cloned from the host *Bacillus* organism using protocols that have previously been used to clone Bt genes, and new synthetic forms of Bt toxins may also be produced.

- 5 A Bt gene useful in the present invention may include a 5' DNA sequence including a sequence of DNA which will allow for the initiation of transcription and translation of a downstream located Bt sequence in a plant. The Bt gene may also comprise a 3' DNA sequence that includes a sequence derived from the 3' non-coding region of a gene that can be expressed in the plant of interest. The Bt
- 10 gene would also include a DNA sequence coding for a toxic Bt polypeptide produced by *Bacillus thuringiensis* or toxic portions thereof or having substantial amino sequence homology thereto. The Bt coding sequence may include: (i) DNA sequences which code for insecticidal proteins that have substantial homology to Bt endotoxins that are active against insect pests of the plant of
- 15 interest, e.g., the HD73 or HD1 Bt sequences; (ii) sequences coding for insecticidally-active segments of the Bt endotoxin polypeptide, e.g., insecticidally active HD73 or HD1 polypeptides truncated from the carboxy and/or amino termini; and/or (iii) a truncated Bt sequence fused in frame with a sequence(s) that codes for a polypeptide that provides some additional advantage
- 20 such as: (a) genes that are selectable, e.g., genes that confer resistance to antibiotics or herbicides, (b) reporter genes whose products are easy to detect or assay, e.g., luciferase or beta-glucuronidase; (c) DNA sequences that code for polypeptide sequences that have some additional use in stabilizing the Bt protein against degradation or enhance the efficacy of the Bt protein against insects, e.g.,
- 25 protease inhibitors and (d) sequences that help direct the Bt protein to a specific compartment inside or outside the plant cell, e.g., a signal sequence.

- To obtain optimum synthesis of the Bt protein in the plant, it may also be appropriate to adjust the DNA sequence of the Bt gene to more resemble the genes that are efficiently expressed in the plant of interest. Since the codon usage
- 30 of Bt genes may be dissimilar to that used by genes that are expressed in the plant of interest, the expression of the Bt gene in plant cells may be improved by the replacement of these codons with those that are more efficiently expressed in plants, e.g., are used more frequently in the plants of interest (See E. Murray et

al., Nucl. Acids Res., 17, 477 (1989)). Such replacement of codons may require the substitution of bases without changing the amino acid sequence of the resulting Bt polypeptide. The Bt polypeptide may be identical in sequence to the bacterial gene or segments thereof. The complete Bt coding sequence, or sections thereof, containing a higher proportion of preferred codons than the original bacterial gene could be synthesized using standard chemical synthesis protocols, and introduced or assembled into the Bt gene using standard protocols, such as site-directed mutagenesis or DNA polymerization and ligation and the like.

Protease inhibitors may also provide insect resistance. For example, use of a protease inhibitor II gene, pinII, from tomato or potato may be useful. Also advantageous is the use of a pinII gene in combination with a Bt toxin gene. Other genes which encode inhibitors of the insects' digestive system, or those that encode enzymes or co-factors that facilitate the production of inhibitors, may also be useful. This group includes oryzacystatin and amylase inhibitors such as those from wheat and barley.

Genes encoding lectins may confer additional or alternative insecticide properties. (Murdock et al., Phytochemistry, 29 85 (1990); Czapla & Lang, J. Econ. Entomol., 83, 2480 (1990) Lectin genes contemplated to be useful include, for example, barley and wheat germ agglutinin (WGA) and rice lectins.

(Gatehouse et al., J Sci Food Agric, 35, 373 (1984))

Genes controlling the production of large or small polypeptides active against insects when introduced into the insect pests such as lytic peptides, peptide hormones and toxins and venoms, may also be useful. For example, the expression of juvenile hormone esterase, directed towards specific insect pests, may also result in insecticidal activity, or perhaps cause cessation of metamorphosis. (Hammock et al., Nature, 344, 458 (1990))

Transgenic plants expressing genes encoding enzymes that affect the integrity of the insect cuticle may also be useful. Such genes include those encoding, for example, chitinase, proteases, lipases and also genes for the production of nikkomycin. Genes that code for activities that affect insect molting, such as those affecting the production of ecdysteroid UDP-glucosyl transferase, may also be useful.

Genes that code for enzymes that facilitate the production of compounds that reduce the nutritional quality of the plant to insect pests may also be useful. It may be possible, for instance, to confer insecticidal activity to a plant by altering its sterol composition. Further embodiments of the invention concern
5 transgenic plants with enhanced lipoxigenase activity.

The present invention also provides methods and compositions useful in altering plant secondary metabolites. One example concerns altering plants to produce DIMBOA which, it is contemplated, will confer resistance to European corn borer, rootworm and several other insect pests. See, e.g., U.S. Patent
10 6,331,880. DIMBOA is derived from indole-related compounds. The present invention provides methods for increasing the content of indole-related compounds like tryptophan within plant cells and tissues. Hence, according to the invention the methods provided herein may also increase the levels of DIMBOA, and thereby increase the resistance of plants to insects.

15 The introduction of genes that can regulate the production of maysin, and genes involved in the production of dhurrin in sorghum, is also contemplated to be of use in facilitating resistance to earworm and rootworm, respectively.

Further genes encoding proteins characterized as having potential insecticidal activity may also be used. Such genes include, for example, the
20 cowpea trypsin inhibitor (CpTI; Hilder et al., Nature, 330, 160 (1987)) which may be used as a rootworm deterrent; genes encoding avermectin (Avermectin and Abamectin., Campbell, W. C., Ed., 1989; Ikeda et al., J Bacteriol, 169, 5615 1987) which may prove useful as a corn rootworm deterrent; ribosome inactivating protein genes; and genes that regulate plant structures. Transgenic
25 plants including anti-insect antibody genes and genes that code for enzymes that can convert a non-toxic insecticide (pro-insecticide) applied to the outside of the plant into an insecticide inside the plant are also contemplated.

Environmental or Stress Resistance or Tolerance

Improvement of a plant's ability to tolerate various environmental
30 stresses can be effected through expression of genes. For example, increased resistance to freezing temperatures may be conferred through the introduction of an "antifreeze" protein such as that of the Winter Flounder (Cutler et al., J Plant Physiol, 135, 351 1989) or synthetic gene derivatives thereof. Improved chilling

- tolerance may also be conferred through increased expression of glycerol-3-phosphate acetyltransferase in plastids (Wolter et al., The EMBO J., **11**, 4685 (1992)). Resistance to oxidative stress can be conferred by expression of superoxide dismutase (Gupta et al., Proc. Natl. Acad. Sci USA, **90**, 1629 (1993)),
- 5 and can be improved by glutathione reductase (Bowler et al., Ann Rev. Plant Physiol., **43**, 83 (1992)).

- It is contemplated that the expression of genes that favorably affect plant water content, total water potential, osmotic potential, and turgor will enhance the ability of the plant to tolerate drought and will therefore be useful. It is
- 10 proposed, for example, that the expression of genes encoding for the biosynthesis of osmotically-active solutes may impart protection against drought. Within this class are genes encoding for mannitol dehydrogenase (Lee and Saier, J. Bacteriol., **258**, 10761 (1982)) and trehalose-6-phosphate synthase (Kaasen et al., J. Bacteriology, **174**, 889 (1992)).

- 15 Similarly, other metabolites may protect either enzyme function or membrane integrity (Loomis et al., J. Expt. Zoology, **252**, 9 (1989)), and therefore expression of genes encoding for the biosynthesis of these compounds might confer drought resistance in a manner similar to or complimentary to mannitol. Other examples of naturally occurring metabolites that are osmotically
- 20 active and/or provide some direct protective effect during drought and/or desiccation include fructose, erythritol, sorbitol, dulcitol, glucosylglycerol, sucrose, stachyose, raffinose, proline, glycine, betaine, ononitol and pinitol. See, e.g., U.S. Patent 6,281,411.

- Three classes of Late Embryogenic Proteins have been assigned based on structural similarities (see Dure et al., Plant Molecular Biology, **12**, 475 (1989)).
- 25 Expression of structural genes from all three LEA groups may confer drought tolerance. Other types of proteins induced during water stress, which may be useful, include thiol proteases, aldolases and transmembrane transporters, which may confer various protective and/or repair-type functions during drought stress.
- 30 See, e.g., PCT/CA99/00219 (Na⁺/H⁺ exchanger polypeptide genes). Genes that effect lipid biosynthesis might also be useful in conferring drought resistance.

The expression of genes involved with specific morphological traits that allow for increased water extractions from drying soil may also be useful. The

expression of genes that enhance reproductive fitness during times of stress may also be useful. It is also proposed that expression of genes that minimize kernel abortion during times of stress would increase the amount of grain to be harvested and hence be of value.

- 5 Enabling plants to utilize water more efficiently, through the introduction and expression of genes, may improve the overall performance even when soil water availability is not limiting. By introducing genes that improve the ability of plants to maximize water usage across a full range of stresses relating to water availability, yield stability or consistency of yield performance may be realized.

10 **Disease Resistance or Tolerance**

Resistance to viruses may be produced through expression of genes. For example, expression of antisense genes targeted at essential viral functions or expression of genes encoding viral coat proteins may impart resistance to the virus.

- 15 Resistance to diseases caused by bacteria and fungi may be conferred through introduction of genes. For example, genes encoding so-called "peptide antibiotics," pathogenesis related (PR) proteins, toxin resistance, and proteins affecting host-pathogen interactions such as morphological characteristics may be useful.

20 **Mycotoxin Reduction/Elimination**

- Production of mycotoxins, including aflatoxin and fumonisin, by fungi associated with plants is a significant factor in rendering grain not useful. Inhibition of the growth of these fungi may reduce the synthesis of these toxic substances and therefore reduce grain losses due to mycotoxin contamination. It may be possible to introduce genes into plants such that would inhibit synthesis of the mycotoxin without interfering with fungal growth. Further, expression of a novel gene which encodes an enzyme capable of rendering the mycotoxin nontoxic would be useful in order to achieve reduced mycotoxin contamination of grain.
- 25

30 **Plant Composition or Quality**

The composition of the plant may be altered, for example, to improve the balance of amino acids in a variety of ways including elevating expression of native proteins, decreasing expression of those with poor composition, changing

- the composition of native proteins, or introducing genes encoding entirely new proteins possessing superior composition. *See, e.g.,* U.S. Patent No. 6,160,208 (alteration of seed storage protein expression). The introduction of genes that alter the oil content of the plant may be of value. *See, e.g.,* U.S. Patent Nos. 5 6,069,289 and 6,268,550 (ACCase gene). Genes may be introduced that enhance the nutritive value of the starch component of the plant, for example by increasing the degree of branching, resulting in improved utilization of the starch in cows by delaying its metabolism.

Plant Agronomic Characteristics

- 10 Two of the factors determining where plants can be grown are the average daily temperature during the growing season and the length of time between frosts. Expression of genes that are involved in regulation of plant development may be useful, e.g., the liguleless and rough sheath genes that have been identified in corn.
- 15 Genes may be introduced into corn that would improve standability and other plant growth characteristics. Expression of genes which confer stronger stalks, improved root systems, or prevent or reduce ear droppage would be of value to the farmer

Nutrient Utilization

- 20 The ability to utilize available nutrients may be a limiting factor in growth of plants. It may be possible to alter nutrient uptake, tolerate pH extremes, mobilization through the plant, storage pools, and availability for metabolic activities by the introduction of genes. These modifications would allow a plant to more efficiently utilize available nutrients. For example, an
- 25 increase in the activity of an enzyme that is normally present in the plant and involved in nutrient utilization may increase the availability of a nutrient. An example of such an enzyme would be phytase.

Male Sterility

- Male sterility is useful in the production of hybrid seed, and male sterility 30 may be produced through expression of genes. It may be possible through the introduction of TURF-13 via transformation to separate male sterility from disease sensitivity. *See Levings, Science, 250:942-947, 1990.* As it may be

necessary to restore male fertility for breeding purposes and for grain production, genes encoding restoration of male fertility may also be introduced.

Selection and Characterization of Resistant Cell Lines

- 5 Selections are carried out until cells or tissue are recovered which are observed to be growing well in the presence of normally inhibitory levels of a tryptophan analog thereof. These cell "lines" are subcultured several additional times in the presence of a tryptophan analog to remove non-resistant cells and then characterized. The amount of resistance that has been obtained is
- 10 determined by comparing the growth of these cell lines with the growth of unselected cells or tissue in the presence of various tryptophan analogs at various concentrations. Stability of the resistance trait of the cultured cells may be evaluated by simply growing the selected cell lines in the absence of the tryptophan analog for various periods of time and then analyzing growth after re-
- 15 exposing the tissue to the analog. The resistant cell lines may also be evaluated using *in vitro* chemical studies to verify that the site of action of the analog is altered to a form that is less sensitive to inhibition by tryptophan analogs.

- Transient expression of an anthranilate synthase gene can be detected and quantitated in the transformed cells. Gene expression can be quantitated by RT-
- 20 PCR analysis, a quantitative Western blot using antibodies specific for the cloned anthranilate synthase or by detecting enzyme activity in the presence of tryptophan or an amino acid analog of tryptophan. The tissue and subcellular location of the cloned anthranilate synthase can be determined by immunochemical staining methods using antibodies specific for the cloned
- 25 anthranilate synthase or subcellular fractionation and subsequent biochemical and/or immunological analyses. Sensitivity of the cloned anthranilate synthase to agents can also be assessed. Transgenes providing for expression of an anthranilate synthase or anthranilate synthase tolerant to inhibition by an amino acid analog of tryptophan or free L-tryptophan can then be used to transform
- 30 monocot and/or dicot plant tissue cells and to regenerate transformed plants and seeds. Transformed cells can be selected by detecting the presence of a selectable marker gene or a reporter gene, for example, by detecting a selectable herbicide resistance marker. Transient expression of an anthranilate synthase

gene can be detected in the transgenic embryogenic calli using antibodies specific for the cloned anthranilate synthase, or by RT-PCR analyses.

Plant Regeneration and Production of Seed

5 Transformed embryogenic calli, meristematic tissue, embryos, leaf discs and the like can then be used to generate transgenic plants that exhibit stable inheritance of the transformed anthranilate synthase gene. Plant cell lines exhibiting satisfactory levels of tolerance to an amino acid analog of tryptophan are put through a plant regeneration protocol to obtain mature plants and seeds
10 expressing the tolerance traits by methods well known in the art (for example, see U.S. Pat. Nos. 5,990,390, 5,489,520; and Laursen et al., Plant Mol. Biol., 24, 51 (1994)). The plant regeneration protocol allows the development of somatic embryos and the subsequent growth of roots and shoots. To determine that the tolerance trait is expressed in differentiated organs of the plant, and not solely in
15 undifferentiated cell culture, regenerated plants can be assayed for the levels of tryptophan present in various portions of the plant relative to regenerated, non-transformed plants. Transgenic plants and seeds can be generated from transformed cells and tissues showing a change in tryptophan content or in resistance to a tryptophan analog using standard methods. It is especially
20 preferred that the tryptophan content of the leaves or seeds is increased. A change in specific activity of the enzyme in the presence of inhibitory amounts of tryptophan or an analog thereof can be detected by measuring enzyme activity in the transformed cells as described by Widholm, Biochimica et Biophysica Acta, 279, 48 (1972). A change in total tryptophan content can also be examined by
25 standard methods as described by Jones et al., Analyst, 106, 968 (1981).

Mature plants are then obtained from cell lines that are known to express the trait. If possible, the regenerated plants are self pollinated. In addition, pollen obtained from the regenerated plants is crossed to seed grown plants of agronomically important inbred lines. In some cases, pollen from plants of these
30 inbred lines is used to pollinate regenerated plants. The trait is genetically characterized by evaluating the segregation of the trait in first and later generation progeny. The heritability and expression in plants of traits selected in

tissue culture are of particular importance if the traits are to be commercially useful.

The commercial value of tryptophan overproducer soybeans, cereals and other plants is greatest if many different hybrid combinations are available for sale. The farmer typically grows more than one kind of hybrid based on such differences as maturity, standability or other agronomic traits. Additionally, hybrids adapted to one part of the country are not adapted to another part because of differences in such traits as maturity, disease, and insect resistance. Because of this, it is necessary to breed tryptophan overproduction into a large number of parental inbred lines so that many hybrid combinations can be produced.

A conversion process (backcrossing) is carried out by crossing the original overproducer line to normal elite lines and crossing the progeny back to the normal parent. The progeny from this cross will segregate such that some plants carry the gene responsible for overproduction whereas some do not. Plants carrying such genes will be crossed again to the normal parent resulting in progeny which segregate for overproduction and normal production once more. This is repeated until the original normal parent has been converted to an overproducing line, yet possesses all other important attributes as originally found in the normal parent. A separate backcrossing program is implemented for every elite line that is to be converted to tryptophan overproducer line.

Subsequent to the backcrossing, the new overproducer lines and the appropriate combinations of lines which make good commercial hybrids are evaluated for overproduction as well as a battery of important agronomic traits. Overproducer lines and hybrids are produced which are true to type of the original normal lines and hybrids. This requires evaluation under a range of environmental conditions where the lines or hybrids will generally be grown commercially. For production of high tryptophan soybeans, it may be necessary that both parents of the hybrid seed be homozygous for the high tryptophan character. Parental lines of hybrids that perform satisfactorily are increased and used for hybrid production using standard hybrid seed production practices.

The transgenic plants produced herein are expected to be useful for a variety of commercial and research purposes. Transgenic plants can be created for use in traditional agriculture to possess traits beneficial to the consumer of

the grain harvested from the plant (e.g., improved nutritive content in human food or animal feed). In such uses, the plants are generally grown for the use of their grain in human or animal foods. However, other parts of the plants, including stalks, husks, vegetative parts, and the like, may also have utility, including use as part of animal silage, fermentation feed, biocatalysis, or for ornamental purposes.

Transgenic plants may also find use in the commercial manufacture of proteins or other molecules, where the molecule of interest is extracted or purified from plant parts, seeds, and the like. Cells or tissue from the plants may also be cultured, grown *in vitro*, or fermented to manufacture such molecules.

The transgenic plants may also be used in commercial breeding programs, or may be crossed or bred to plants of related crop species. Improvements encoded by the recombinant DNA may be transferred, e.g., from soybean cells to cells of other species, e.g., by protoplast fusion.

In one embodiment, a transgene comprised of a maize anthranilate α -domain isolated from a maize cell line tolerant to 5-MT and linked to the 35S CaMV promoter is introduced into a 5-MT sensitive monocot or dicot tissue using microprojectile bombardment. Transformed embryos or meristems are selected and used to generate transgenic plants. Transformed calli and transgenic plants can be evaluated for tolerance to 5-MT or 6-MA and for stable inheritance of the tolerance trait.

The following examples further illustrate the invention and are not intended to be limiting thereof.

EXAMPLE 1: Isolation and *E. coli* Expression of Anthranilate Synthase from *Agrobacterium tumefaciens*.

This example describes the isolation of anthranilate synthase from *Agrobacterium tumefaciens* and its expression in *E. coli*.

Cloning of *Agrobacterium tumefaciens* AS

The nucleotide and amino acid sequences of the anthranilate synthase coding region from *Rhizobium meliloti* (GenBank accession number: P15395)

was used to search an *Agrobacterium tumefaciens* C58 genomic sequence database (Goodner et al. Science 294, 2323-2328 (2001)). The search consisted of tblastn using blosum62 matrix, (Altschul et. al., Nucleic Acid Res., 25, 3389-3402 (1997)).

- 5 The identified AS homolog in the *Agrobacterium tumefaciens* C58 genomic sequence database was cloned by PCR using genomic DNA from *Agrobacterium tumefaciens* strain C58 (ATCC No. 33970) as the template. The primary PCR reaction was carried out using the following primers:

5'-TTATGCCGCCTGTCATCG-3' (SEQ ID NO:47) and

- 10 5'-ATAGGCTTAATGGTAACCG-3' (SEQ ID NO:48).

Gene amplification parameters were as follows: (a) denature at 95°C for 30 seconds, (b) anneal at 50°C for 30 seconds and (c) extend at 72 °C for 2 minutes, using Expand high fidelity PCR (Roche Biochemicals), according to manufacturer directions.

- 15 An additional round of PCR amplification, yielding a product of approximately 2.3 Kb in length, was carried out using the amplified template from above and the following nested primers:

5'-CTGAACAACAGAAGTACG-3' (SEQ ID NO:49)

5'-TAACCGTGTCATCGAGCG-3' (SEQ ID NO:50).

- 20 The purified PCR product was ligated into pGEM-T easy (Promega Biotech) resulting in the plasmid pMON61600 (Figure 1). pMON61600 was sequenced using standard sequencing methodology. Confirmation of the correct sequence was obtained by comparison of the sequence the *Rhizobium meliloti* anthranilate synthase sequence (Figure 2). The translated amino acid sequence
25 from the isolated clone (SEQ ID NO:4) shared 88% identity with the *Rhizobium meliloti* enzyme (SEQ ID NO:7) (Figure 2).

The abbreviation "AgroAS" or *A. tumefaciens* AS is sometimes used herein to refer to *Agrobacterium tumefaciens* anthranilate synthase.

30 ***E. coli* expression of *Agrobacterium tumefaciens* AS**

The following vectors were constructed to facilitate subcloning of the *Agrobacterium tumefaciens* AS gene into a suitable expression vector.

A 2215 base pair PCR fragment was generated using pMON61600 as the template and the following primers:

5'-AAAAAGATCTCCATGG TAACGATCATTCAAGG-3' (SEQ ID NO:51)

5'-AAAAGAA TTCTTATCACGCGGCCTTGGTCTTCGCC-3' (SEQ ID

5 NO:52).

The plasmid pMON61600 was digested with restriction enzymes NcoI and RsrII. In addition, a 409bp fragment (derived by digesting the 2215 base pair PCR product with NcoI and RsrII) was then ligated into the digested pMON61600 plasmid, thereby replacing the NcoI/RsrII fragment, and resulting
10 in a NcoI site in frame with the translation initiation codon (ATG) of *Agrobacterium tumefaciens* AS to yield plasmid pMON34692 (Figure 3).

The base T7 *E. coli* expression plasmid, pMON34697 (Figure 4), was generated by restriction digestion of pET30a (Novogen, Inc) with SphI and BamHI. The resulting 4,969 bp fragment was purified and subcloned with a 338
15 bp SphI and BamHI fragment from pET11d (Novogen, Inc).

The plasmid pMON34705 (Figure 5) was generated by restriction digestion of pMON34697 with NcoI and SacI. The resulting 5,263 bp fragment was then purified and ligated with a 2,256 bp NcoI and SacI fragment from pMON34692 containing *Agrobacterium tumefaciens* AS.

The plasmid pMON34705 was transformed into *E. coli* BL21(DE3) (F-
20 *ompT HsdS_B(r_B⁻m_B)gal dcm* (DE3)) according to manufacturer's instructions (Novogen, Inc). DE3 is a host lysogen of λ DE3 containing chromosomal copy of T7 RNA polymerase under control of an isopropyl-1-thio-D-galactopyranoside (IPTG) inducible *lacUV5*.

25 Transformed cells were selected on kanamycin plates that had been incubated at 37°C overnight (10 hours). Single colonies were transferred to 2mL of LB (Luria Broth; per liter, 10g tryptone, 5g yeast extract, 10g NaCl, and 1g glucose (optional)) or 2X-YT broth (per liter, 16g tryptone, 10g yeast extract, 5g NaCl) and then placed in a 37°C incubator and shaken at 225rpm for 3 hours.
30 The cells were removed and 4μL of 100mM IPTG was added to the culture and returned to the 37°C incubator for an additional 2 to 3 hours. A 1mL aliquot of the cells was removed and sonicated in sonication buffer, (50mM potassium phosphate (pH 7.3), 10% glycerol, 10mM 2-mercaptoethanol and 10mM MgCl₂).

The resulting lysed cell extract was the source material for the standard AS assay described below. The results established that the expression system based on plamid pMON34705 was able to produce soluble and enzymatically active *Agrobacterium tumefaciens* AS protein that accounts for approximately 50% of

5 total soluble extracted protein.

EXAMPLE 2: High Trp Seed Levels are Achieved by Transformation of Plants with Wild Type *Agrobacterium* Anthranilate Synthase

Expression Vector pMON58120

5 The vector pMON58120 (Figure 34) encodes a fusion between a 264 base pair *Arabidopsis* small subunit (SSU) chloroplast targeting peptide (CTP, SEQ ID NO:71) and a 2187 base pair wild type *Agrobacterium* anthranilate synthase (AgroAS) open reading frame (SEQ ID NO:1). See, Stark et al., (1992) Science 258: 287. Expression of this open reading frame is driven by the soy 7S
10 alpha prime (7S α') promoter.

 Upon translation on cytoplasmic ribosomes, the fusion (immature protein) is imported into chloroplast where the chloroplast targeting sequence is removed. There are two cleavage sites in the CTP1. The first site is 30 base pairs upstream of the CDS start (C/M), and the other is at the initial methionine
15 (C/M). The second cleavage site does not seem to be processed efficiently. The cleavage is predicted to yield a mature protein of about 70Kd that has AS activity as shown by enzyme activity data and trp efficacy data.

 The AS gene was transformed with the synthetic CP4 gene that confers glyphosate resistance, however the CP4 gene is processed separately from the AS
20 gene. Expression of the CP4 gene was driven by the FMV promoter, which is a 35S promoter from Figwort Mosaic Virus. Glyphosate resistance allows for selection of the transformed plants.

Western analysis of AS protein

25 Thirty-five transformation events of pMON58120 were analyzed for AgroAS protein presence. AgroAS protein was detected with a polyclonal antibody raised in rabbits against purified His-tagged AgroAS. The His-tagged, full-length Agro-AS polypeptide was used as an antigen to generate a population of polyclonal antibodies in rabbits by CoCalico Biological, INC. The
30 recombinant His-tagged Agro-AS DNA was placed into a pMON 34701 (pet-30a-agroAS) expression vector. The His-AgroAS fusion protein was expressed in *E.coli* BL21(DE3) and purified by Ni-NTA resin system (Qiagen protocol). For western analysis, primary rabbit anti-AgroAS antibodies were used at

1:5,000 dilution. Secondary, goat anti-rabbit alkaline phosphatase-conjugated antibodies were used at 1:5,000 dilution. In transgenic lines carrying 7Salpha'-Agro AS genes, western blot analysis consistently revealed the presence of a single band that specifically cross-reacted with anti-AgroAS antibodies. This band was not detected in the nontransgenic control line.

Free Amino Acid Analysis of Soy and Arabidopsis Seed

Amino Acid Extraction: About 50 mg of crushed soy seed (5 mg of *Arabidopsis*) material was placed in each centrifuge vial. One milliliter of 5% trichloroacetic acid was added to each sample (100 μ l for *Arabidopsis*). The samples were vortexed, and allowed to sit, with agitation, at room temperature for 15 min. They were then microcentrifuged for 15 min at 14000 rpm. Some of the supernate was then removed, placed in a HPLC vial and sealed. Samples were kept at 4°C in the analysis queue.

Amino Acid Analysis: The reagents utilized for amino acid analysis included the OPA reagent (o-phthalaldehyde and 3-mercaptopropionic acid in borate buffer (Hewlett-Packard, PN 5061-3335)) where the borate buffer (0.4 N in water, pH 10.2). The analysis was performed using the Agilent 1100 series HPLC system as described in the Agilent Technical Publication, "Amino Acid Analysis Using Zorbax Eclipse-AAA Columns and the Agilent 1100 HPLC." March 17, 2000. First, 0.5 μ l of the sample was derivatized with 2.5 μ l of OPA reagent in 10 μ l of borate buffer. Second, the derivative is injected onto a Eclipse XDB-C18 5 μ m, 4.6 x 150 mm column using a flow rate of 1.2 ml/min. Amino acid concentrations were measured using fluorescence: excitation at 340 nm, emission at 450 nm. Elution was with a gradient of HPLC Buffers A and B according to Table A, where HPLC Buffer A was 40 mM Na₂HPO₄, pH=7.8 and HPLC Buffer B was 9 : 9 : 2 :: Methanol : Acetonitrile : Water.

Table A: Amino Acid Elution

Time	0	20	21	26	27
% Buffer B	5	65	100	100	100

Amino acid standards were prepared from the dry chemicals, using all amino acids of interest. Proline analysis required an additional derivatization step with 9-fluorenylmethyl-chloroformate (FMOC). Amino acid standards were also sometimes purchased in concentrations ranging from 0 to 100 $\mu\text{g/ml}$. Samples were reported in $\mu\text{g/g}$ of seed powder. Calculations were performed using an MS Excel spreadsheet found on Mynabird TMBROW > Public > Calculators > External Standard.xls.

Expression of Wild Type *Agrobacterium* Anthranilate Synthase in *Arabidopsis*.

The vector pMON 58120 was transformed into *Arabidopsis* plants by vacuum infiltration of the secondary inflorescences, and plants were allowed to set transgenic seed. The seed was collected and screened for the presence of a selectable marker (glyphosate resistance). Glyphosate resistant plants were grown to maturity and seed from each plant, which was designated a transformation event, and analyzed for tryptophan content (Table B). Selected transformation events were also analyzed for the presence of the expressed *Agrobacterium* anthranilate synthase protein in the mature seed by Western blot analysis as shown in Table B.

Table B: Analysis of Transformants

Transformation Event	Trp (ppm)	Protein present
7317	2547	+
7315	2960	+
7319	3628	+
7313	3979	+

Expression of Wild Type *Agrobacterium* Anthranilate Synthase in Soy (Glycine Max)

Thirty-three out of thirty-five soy transformation events analyzed had an increase in seed trp levels, for example, from above 500 ppm and up to 12,000 ppm. In nontransgenic soy seeds, the trp level is less than 200 ppm. All seeds that contained high amounts of trp demonstrated anthranilate synthase protein

expression by western blotting. Table C presents data for nineteen soy events that contain high trp levels and also are positive for anthranilate synthase anthranilate synthase protein by western blot analysis.

Table C: Correlation between the Presence of the Agro AS Protein and Tryptophan Levels in Nineteen Soy Transgenic Events bearing pMON58120

Pedigree	Trp max (ppm)	Trp average (ppm)	Protein present ?
A3244 (ctr)	306	96	NO
GM_A20380:@.	6444	2246.4	YES
GM_A20532:@.	6055	2556.6	YES
GM_A22043:@.	10422	2557.2	YES
GM_A20598:@.	8861	2859.9	YES
GM_A20744:@.	7121	3373.3	YES
GM_A20381:@.	6392	3572.9	YES
GM_A20536:@.	9951	3581.5	YES
GM_A20510:@.	8916	3592.7	YES
GM_A20459:@.	8043	3900.4	YES
GM_A20337:@.	7674	4088.6	YES
GM_A20533:@.	9666	4183.2	YES
GM_A20577:@.	6276	4434.1	YES
GM_A20339:@.	9028	4687.8	YES
GM_A20386:@.	8487	5285.3	YES
GM_A20457:@.	11007	5888.9	YES
GM_A20379:@.	7672	6416.1	YES
GM_A20537:@.	9163	6695.8	YES
GM_A20534:@.	12676	7618.2	YES
GM_A20576:@.	10814	7870.1	YES

The Agro AS enzyme assay

The specific activity of anthranilate synthase was measured in eleven transformation events carrying the pMON58120 construct. Individual soybean immature seeds were analyzed using an HPLC-based end-point assay based on the method described by C. Paulsen (J. Chromatogr. **547**, 1991, 155-160). Briefly, desalted extracts were generated from individual seeds in grinding buffer (100mM Tris pH7.5, 10% glycerol, 1mM EDTA, 1mM DTT) and incubated for 30 min with reaction buffer (100mM tris pH 7.5, 1 mM chorismate, 20mM glutamine, and 10mM MgCl₂). Agro AS activity was measured in the presence or absence of 25mM trp. The reaction was stopped with phosphoric acid and the

amount of anthranilate formed was quantified by HPLC using a fluorescence detector set at 340nm/excitation and 410 nm/emission.

The specific activity of AS in immature segregating transgenic seeds ranged from 1.5-fold up to 70-fold increase compared to a nontransgenic control, reaching as high as 6,000 pmoles/mg/min. As shown in the last column of Table D, the anthranilate synthase activity in transgenic plants is resistant to tryptophan inhibition (see Table D).

Table D: Agro AS Enzyme Activity in Transgenic Event 20576

Event	Seed No.	Specific Activity (pmoles/mg/min)	Specific Activity (pmoles/mg/min) (+ 25 micromolar Trp)
Control	3244-1	95.4	42.4
Control	3244-2	85.5	40.6
20576	20576-1	6060.2	4407.1
20576	20576-2	3783.8	1709.4
20576	20576-3	2768.3	2431.7
20576	20576-4	4244.08	2125.2

10

EXAMPLE 3: Soybean Transformation with a Vector

Containing a Maize Anthranilate Synthase α -Subunit gene.

The coding sequence for a maize anthranilate synthase α -subunit was isolated from pMON52214 (Figure 22) by digesting with XbaI in combination with a partial NcoI digest (*see* Anderson et. al. U.S. Patent 6,118,047). The resulting 1952 bp DNA fragment representing the anthranilate synthase α coding region was gel purified, and the ends were made blunt. The plasmid pMON53901 (Figure 23) was digested with BglII and EcoRI, to generate a 6.8 Kb fragment. After isolation, the ends of the 6.8 Kb fragment were made blunt and dephosphorylated. The 1952 Kb fragment containing the AS α gene was then ligated into the blunt-ended 6.8 kb pMON53901 fragment to generate pMON39324, a maize 7SP-AS α -NOS expression vector (Figure 24).

This pMON39324, a maize 7SP-AS α -NOS cassette was subsequently digested with BamHI resulting in a 2.84 Kb DNA fragment, containing the 7S promoter and maize AS α coding sequence. The plasmid pMON39322 (Figure 25) was digested with BamHI resulting in a 5.88 kb DNA fragment. These two

fragments were then ligated together to create pMON39325 (Figure 26), a transformation vector containing 7S promoter-maize AS α -NOS terminator cassette subcloned into pMON39322.

Using similar procedures, the coding sequence for a maize anthranilate
5 synthase α -subunit was cloned downstream from the USP promoter to generate a
pMON58130 expression vector, downstream from the Arc5 promoter to generate
a pMON69662 expression vector, downstream from the Lea9 promoter to
generate a pMON69650 expression vector, and downstream from the Perl
promoter to generate a pMON69651 expression vector. A list with these
10 expression vectors is presented in Table E.

Table E: C28-Maize Anthranilate Synthase Constructs

Seed Generation	Expression Cassette	Vector Name
R4	7Sa'-maize-AS α	PMON39325
R2	Napin-maize-AS α	PMON58023
R1	USP-maize-AS α	PMON58130
R1	Arc5-maize-AS α	PMON69662
R1	Lca9-maize-AS α	PMON69650
R1	Per1-maize-AS α	PMON69651

These vectors were used for plant transformation and propagation

- 5 experiments. Soybean plants were transformed with the maize AS-containing vectors using the microprojectile bombardment technology as described herein. Several transgenic soybean lines were established for each type of vector and propagated through the number of generations indicated in Table E.

- For example, three homozygous lines were established that carried the
- 10 7Salph'-maize-AS transgene from pMON39325. These three lines were grown in a randomized block design in two different locations. Mature seed was produced and analyzed for free amino acid content. Controls were included to establish baseline trp levels, i.e. the three corresponding negative isolines and the nontransgenic controls.

- 15 Table F provides R4 seed tryptophan in ppm for pMON39325 transformant and control lines, showing that the average non-transgenic soybeans contain about 100-200 μ g tryptophan/g seed powder whereas the pMON39325 transformants contain substantially more Trp. *See also* Figure 27.

**Table F: Trp Levels in seeds of Soybean Plants Transformed
with the C28 *Zea mays* mutant (pMON39325)**

Positive isoleine number	Average trp of Positive Isoleine (ppm)	Standard deviation	Average trp of corresponding Negative isoleine (ppm)	Standard deviation
39325-1	3467	377	226	55
35325-2	2623	307	164	20
35325-3	3715	152	184	64
35325-4	2833	165	202	146
35325-5	3315	161	173	34
35325-6	2394	318	144	22
nontransgenic control-7			191	24
nontransgenic control-8			118	23

Five other constructs, expressing the C28 maize anthranilate synthase under the control of five different promoters (Table E) were transformed into soy and transgenic plants were obtained. Each construct generated events high in trp. An example illustrating events generated by Per1-C28 maize anthranilate synthase is shown in Tables G and H.

**Table G: C28 maize AS Protein Expression Correlates
with Increased Trp Levels in Three Transgenic Events
bearing Per1-C28 maize AS (pMON69651)**

Pedigree	Trp average (ppm)	Protein present ?
Control	96	NO
22689	2375	Yes
22787	1707	Yes
22631	1116	Yes

Table H illustrates the enzymatic activity of C28 maize AS in R1 seeds from soybean plants transformed with the pMON69651 expression vector.

**Table H: Specific Activity of C28 maize AS in R1 Seeds
of pMON69651 Transformants**

Event	Seed number	Specific activity (pmoles/mg/min)	Specific activity (pmoles/mg/min) (+ 25 micromolar tryptophan)
-------	-------------	-----------------------------------	---

Control		51.6	2.6
22689	22689-1	130.9	64.7
	22689-2	115.3	
	22689-3	148.5	61.1
	22689-4	149.5	
	22698-5	133.8	60.3

These results indicate that there is a substantial increase in tryptophan when soybean plant tissues are transformed with the C28 maize AS gene.

- 5 The high trp levels shown in Table G correlate with the presence of the AS protein and with increased specific activity (2.5 fold higher than in nontransgenic controls) for the transgenic enzyme (Table H). As shown in Table H - and as predicted by the biochemical properties of the C28 maize AS enzyme - the specific activity of transgenic events is tryptophan-resistant.

10

EXAMPLE 4: Rational Design of *Agrobacterium tumefaciens* Anthranilate Synthase tryptophan feedback insensitive mutants.

This example describes vectors containing mutant *Agrobacterium tumefaciens* anthranilate synthase enzymes that have various degrees of sensitivity or insensitivity to feedback inhibition by tryptophan or tryptophan analogs.

15

Generation of *Agrobacterium tumefaciens* Mutant Anthranilate Synthase Genes.

20

Using protein structural information from *Sulfolobus solfataricus* anthranilate synthase as a guide (Knochel et. al., Proc. Natl. Acad. Sci. USA, 96, 9479-9484 (1999)) several *Agrobacterium tumefaciens* anthranilate synthase mutants were rationally designed utilizing protein informatics to confidently assign several residues involved in tryptophan binding. This was accomplished by alignment of the *Agrobacterium tumefaciens* anthranilate synthase gene with the anthranilate synthase amino acid sequence from *Sulfolobus solfataricus* (Figure 6). The putative tryptophan binding and catalysis regions of the *Agrobacterium tumefaciens* were assigned by combining the knowledge of the

25

structural information with the sequence homology. Residues in the binding pocket were identified as potential candidates for altering to provide resistance to feedback inhibition by tryptophan.

Based on the structural analysis of the *Sulfolobus solfataricus* anthranilate synthase enzyme, it suggested that amino acids E30, S31, I32, S42, V43, N204, P205, M209, F210, G221, and A373 were involved in tryptophan binding. Based on the pairwise alignment, N204, P205, and F210 of *Sulfolobus solfataricus* were also conserved in the monomeric *Agrobacterium tumefaciens* anthranilate synthase as residues N292, P293, and F298 respectively.

However, due to multiple insertions and deletions, the N-terminal regions of the *Sulfolobus solfataricus* and *Agrobacterium tumefaciens* enzymes were highly divergent. For this reason, it was necessary to manually assign residues at the N-terminal region of the *Agrobacterium tumefaciens* anthranilate synthase involved in tryptophan regulation (Figure 6). Structural analysis indicated that the motif "LLES" formed a β sheet in the tryptophan-binding pocket. This structure appeared to be highly conserved among the heterotetrameric enzymes. The known monomeric enzymes were then manually aligned to the *Sulfolobus solfataricus* sequence using the "LLES" motif as a landmark (Figure 21). Based on this protein informatics analysis, amino acid residues V48, S50, S51, and N52 in *Agrobacterium tumefaciens* AS were also likely to be involved in tryptophan binding.

With the putative tryptophan binding residues assigned in the *Agrobacterium tumefaciens* monomeric enzyme, several distinct strategies were rationalized for reducing the sensitivity of the enzyme to tryptophan inhibition. These substitutions included for example, enlarging the tryptophan-binding pocket (F298A), narrowing the binding pocket (V48F, V48Y, S51F, S51C, N52F, F298W), increasing the polarity of the binding pocket (S50K), or distorting the shape of the binding pocket by changing the protein main chain conformation (P293A, P29G).

***A. tumefaciens* AS site-directed mutagenesis**

Site directed mutagenesis was used to generate ten single amino acid substitutions six sites. The mutations were introduced into the *Agrobacterium*

tumefaciens AS in pMON34705 using the QuikChange™ Site-Directed Mutagenesis Kit (Stratagene). The primers used for site directed mutagenesis were SEQ ID NO:9-42 (Figure 7; F = forward, R = reverse). Each primer sequence is specific for alteration of the nucleic acid at a specific location in the sequence and thus changing the encoded codon to code for a new amino acid. For example, S51C designates a change from serine to cysteine at amino acid position 51 in the *Agrobacterium tumefaciens* AS peptide sequence.

Following mutagenesis the sequence of the entire gene was reconfirmed and the variants expressed and purified from *E. coli* as described below for the wild type enzyme. The resultant plasmids comprising mutant *Agrobacterium tumefaciens* AS are suitably cloned into a plasmid for overproduction of protein using the T7 expression system as described in Example 1.

***Agrobacterium tumefaciens* AS protein expression and purification**

Agrobacterium tumefaciens AS wild type and mutant enzymes were expressed in *E. coli* as described in Example 1. The purification of all the *Agrobacterium tumefaciens* AS enzymes, including wild type and mutants thereof, was performed at 4 °C. The cells (approximate wet weight of 1g) were suspended in 20 ml of purification buffer (50 mM potassium phosphate, pH 7.3, 10 mM MgCl₂, 10 mM 2-mercaptoethanol, 10% glycerol) and lysed by ultrasonication (Branson sonifier Cell Disruptor, W185). Supernatant was collected after centrifugation of the homogenate at 20,000 x g for 15 min. The supernatant was subjected to ammonium sulfate fractionation (30 to 65% saturation). The precipitate was collected after centrifugation at 20,000 x g for 15 min and dissolved in 3 ml of the purification buffer and then loaded as a whole on an Econo-Pac 10DG desalting column, pre-equilibrated with the same buffer. Fractions containing the enzyme were detected by the developed assay and pooled. The pooled enzyme (4.3mls) was loaded on a 10 ml DEAE Sephacel (Pharmacia Biotech) column (1.5 x 7.5 cm) equilibrated with the same buffer. The column was washed with 30 ml of the purification buffer and the enzyme was eluted with 30 ml of 50 mM NaCl in the same buffer. Fractions containing high AS activity were pooled and precipitated by 65% ammonium

sulfate saturation and isolated and desalted as above. Fractions containing the enzyme were pooled and stored at -80°C .

Anthranilate synthase enzyme assay and kinetic analysis.

- 5 The standard assay for *Agrobacterium tumefaciens* AS was performed at 25°C in an assay buffer containing 100mM potassium phosphate, pH 7.0, 10mM MgCl_2 , 1mM dithiothreitol, 200 μM chorismate and 10mM L-glutamine. The reaction was started by adding 30 μl of enzyme to the reaction mixture and mixing. The formation of anthranilate was directly monitored by the absorbance
- 10 increase at 320m for 3min. Initial rate of reaction was calculated as unit absorbance increase per second based on the slope of the absorbance change over the reaction time. K_m for chorismate (K_m^{Cho}) was determined in the total volume of 1 ml assay buffer containing 100mM potassium phosphate, pH 7.0, 10mM MgCl_2 , 1mM dithiothreitol with 10mM L-glutamine and varying the
- 15 concentration of chorismate between 2.5-100 μM chorismate. The K_m for glutamine (K_m^{Gln}) was determined in the total volume of 1ml assay buffer containing 100mM potassium phosphate, pH 7.0, 10mM MgCl_2 , 1mM dithiothreitol with 200 μM chorismate and varying the concentration of L-glutamine between 0.1-2mM L-glutamine. IC_{50} for tryptophan ($\text{IC}_{50}^{\text{Tnp}}$) was
- 20 determined with in the total volume of 1ml assay buffer containing 100mM potassium phosphate, pH 7.0, 10mM MgCl_2 , 1mM dithiothreitol, 10mM L-glutamine, 200 μM chorismate and varying the concentration of L-tryptophan between 0.1-10mM L-tryptophan. Kinetic parameters and IC_{50} of AS were calculated after fitting the data to a non-linear regression program (GraFit).
- 25 Several mutants demonstrated reduced sensitivity to tryptophan inhibition while still maintaining enzymatic activity comparable to the wild type enzyme (Table I). These results demonstrate that the extent of sensitivity to tryptophan inhibition can be decreased, for example, by mutating amino acids in the tryptophan-binding pocket of anthranilate synthase and by optimizing of the
- 30 mutations demonstrating feedback insensitivity.

**Table I: Anthranilate Synthase Activity and Effect of Tryptophan
on *Agrobacterium tumefaciens* AS Mutants**

Mutation	Codon	K_m^{Cho} (μM)	K_m^{Gln} (mM)	k_{cat} (s^{-1})	$k_{\text{cat}}/K_m^{\text{Cho}}$ ($\mu\text{M}^{-1}\text{s}^{-1}$)	$\text{IC}_{50}^{\text{Trp}}$ (μM)
WT		8.0	0.11	0.43	5.37×10^{-2}	5
V48F	TTT	4.5	0.08	0.24	5.33×10^{-2}	150
V48Y	TAT	4.2	0.10	0.18	4.28×10^{-2}	650
S50K	AAG	13	0.01	0.13	1.00×10^{-2}	0.1
S51F	TTC	10	0.06	0.08	0.80×10^{-2}	>32,000
S51C	TGC	2.8	0.08	0.15	5.36×10^{-2}	1,500
N52F	TTC	5.5	0.04	0.21	3.82×10^{-2}	41
P293A	GCG	24	0.16	0.35	1.46×10^{-2}	14
P293G	GGG	33	0.07	0.48	1.45×10^{-2}	17
F298A	GCC	9.2	0.10	0.46	5.00×10^{-2}	5.5
F298W	TGG	18	0.14	0.44	2.44×10^{-2}	450

5

**EXAMPLE 5: Random mutagenesis of *Agrobacterium tumefaciens* AS
to generate tryptophan feedback insensitive mutants.**

In addition to the rational design approaches described in Example 4,
other strategies to generate feedback insensitive mutants of anthranilate synthase
include, but are not limited to, random mutagenesis. Random mutagenesis of
the *Agrobacterium tumefaciens* AS, can be accomplished, for example, by
chemical mutagenesis (isolated DNA or whole organism), error prone PCR, and
DNA shuffling. This example describes the use of chemical mutagenesis
followed by genetic selection. The genetic selection approach is also useful for
selection of desirable mutants derived from other mutagenesis techniques.

Generation of *E. coli* expression plasmid containing *A. tumefaciens* AS

The open reading frame from the *Agrobacterium tumefaciens* AS clone
pMON61600 (SEQ ID NO:1, described in Example 1) was amplified by PCR

using primers that contain an Nco 1 site on the 5' end of the forward primer and an Xba1 site on the 3' end of the reverse primer:

5'-CATCCCATGGATGGTAACGATCATT CAGGAT-3' (SEQ ID NO:55); and

5'-GATGCTCTAGAGACAC TATAGAATACTCAAGC-3' (SEQ ID NO:56).

- 5 The resulting PCR product was ligated into pMON25997 (Figure 28), which had the bktB open reading frame (Slater et al., *J. Bact.* 180, p1979-1987 (1998)) removed by digestion with BspH1 and Xba1 resulting in plasmid pMON62000 (Figure 29). pMON62000 is the base plasmid used for mutagenesis and complementation of the tryptophan auxotroph (EMG2ΔtrpE).

10

Generation of an *E. coli* tryptophan auxotroph EMG2ΔtrpE.

- E. coli* strain Ec-8 (EMG2ΔtrpE) was constructed using the suicide vector pKO3 to delete 1,383 base pairs from the chromosomal *trpE* gene of *E. coli* strain EMG2(K-12 wt F+) (*E. coli* Genetic Stock Center). Two amplicons from *E. coli* genomic DNA were PCR amplified. The first amplicon was approximately 1.5kb and contained the first 30bp of the *trpE* ORF at the 3' end. This amplicon contains a BamH1 site at the 5' end and an EcoR1 site at the 3' end. The second amplicon was approximately 1kb and contained the last 150 bp of the *trpE* ORF at the 5' end. This amplicon contains an EcoR1 site at the 5' end and a SalI site at the 3' end. The two amplicons were digested with the appropriate enzymes and ligated together at the EcoR1 site to create an in-frame deletion of *trpE*. Figure 30 shows the resulting sequence of the truncated gene (SEQ ID NO:46). The *trpE* deletion amplicon was ligated into pKO3 at the BamH1 and SalI sites. Gene disruption was performed as described in A. J. Link et al. *J. Bacteriol.*, 179, 6228 (1997).
- 15
- 20
- 25

Complementation of *E. coli* tryptophan auxotroph EMG2ΔtrpE with pMON62000

- E. coli* strain Ec-8 (EMG2ΔtrpE) was transformed with pMON62000 and plated on M9 minimal medium to determine if the deletion was complemented by the addition of pMON62000. A plasmid control (minus the *Agrobacterium tumefaciens* AS insert) and a strain control Ec-8 were also plated onto M9 minimal medium and onto M9 minimal medium with 40μg/ml tryptophan.
- 30

Growth of strain Ec-8 transformed with pMON62000 was observed on M9 without tryptophan, no growth of either of the controls was observed, indicating complementation of the *trpE* deletion in strain Ec-8 by pMON62000.

5 **Hydroxylamine mutagenesis of pMON62000 and genetic selection of mutants**

To generate mutants of anthranilate synthase, pMON62000 was mutated with the chemical mutagen hydroxylamine. The following ingredients were combined in an eppendorf tube: 20µg pMON62000 plasmid DNA and 40µl 2.5 M hydroxylamine, pH 6.0. The volume was brought to a volume of 200µl with 0.1M NaH₂PO₄, pH6.0 + 5mM EDTA, pH 6.0. The tube was incubated at 70°C. After 1.5 hours, 100µl of reaction mixture was dialyzed on a nitrocellulose filter that was floating on approximately 500ml H₂O. After 15 minutes, the DNA was concentrated using Qiagen PCR Purification Kit. After 3 hours, the remaining 100µl of the reaction mixture was removed and purified in the same manner.

E. coli strain Ec-8 was then transformed by electroporation with 100ng of pMON62000 that had been mutagenized for either 1.5 or 3 hours with hydroxylamine. Two transformation procedures were performed for each time point. Transformed cells were allowed to recover for 4 or 6 hours in SOC medium (20g/L Bacto-Tryptone, 5g/L Bacto Yeast Extract, 10ml/L 1M NaCl, 2.5ml/L 1M KCl, 18g glucose).

Two 245mm square bioassay plates were prepared containing M9 minimal medium, plus 2% agar, and 50µg/ml 5-methyl-DL-tryptophan (5-MT). An aliquot of 900 µl of the 1.5 hour mutagenized transformation mixture was plated onto one 50µg/ml 5-MT plate. The remaining 100 µl was plated onto the M9 control plate. The same procedure was performed for the transformation mixture containing the 3.0 hour mutagenized plasmid.

The plates were then incubated at 37°C for approx. 2.5 days. Resistant colonies were isolated from the 5-MT plates and were streaked onto LB-kanamycin (50µg/ml) plates to confirm the presence of the plasmid. All of the selected colonies grew on these plates. Individual colonies from each of the resistant clones were prepped in duplicate to isolate the plasmid. Restriction

digests and PCR were performed and confirmed that all the clones contained the desired *Agrobacterium tumefaciens* AS insert.

The rescued plasmids were then transformed back into strain Ec-8. One colony from each transformation was purified by streaking onto new LB-

- 5 Kanamycin plates. To confirm resistance to 5-MT, individual purified colonies were streaked onto plates containing M9 plus 50 $\mu\text{g/ml}$ 5-MT and 2% agar, and then grown at 37°C for 3 days. Resistance was confirmed for most of the clones. To determine if resistant mutants would remain resistant at an even higher concentration of 5-MT, they were plated onto M9 plus 300 $\mu\text{g/ml}$ 5-MT and 2%
 - 10 Agar. Most clones demonstrated resistance at this high concentration also.

The plasmids from all of the resistant clones were isolated and sequenced on both strands. Some of the mutations from this experiment are diagrammed in Table J.

15 **Table J: *A. tumefaciens trpEG* Sequence Variations
in 5-MT Resistant Clones.**

Database Clone #	Original Clone #	Determined Sequence Variations	K_m^{cho} (μM)	$\text{IC}_{50}^{\text{trp}}$ (μM)
Wt			8.0	5.0
Ec-12	1	G4A Val2Ile		
Ec-18	8	C35T Thr12Ile	15	2.5
Ec-19	9	C2068T Pro690Ser	5.0	3.4
Ec-20	11	G1066A Glu356Lys & C1779T Ile593Ile		

- As indicated by the data in Table J, several mutants had little effect on the K_m and IC_{50} of the mutant enzyme, indicating that these mutations are likely
- 20 not the source of resistance to tryptophan feedback inhibition. For example, the mutation of C to T at nucleotide 35, which changes a threonine residue to isoleucine at amino acid position 12 (Thr12Ile), gives rise to a minor change in K_m^{cho} and $\text{IC}_{50}^{\text{trp}}$ values. Similarly, a change of C to T at nucleotide position 2068, which changes a proline to a serine also gives rise to a minor change in
 - 25 K_m^{cho} and $\text{IC}_{50}^{\text{trp}}$ values. These mutations may therefore, may be "silent"

mutations that give rise to variant gene products having enzymatic properties like those of wild type.

EXAMPLE 6: High Tryptophan Transgenic Soybean Plants.

5 This example sets forth preparation of transgenic soybean plants having elevated tryptophan levels resulting from transformation with tryptophan feedback insensitive mutants of anthranilate synthase from *Agrobacterium tumefaciens*.

10 Vector Construction

Plasmid pMON34711, which harbors the anthranilate synthase clone from *Agrobacterium tumefaciens* containing the F298W mutation described in Example 4, was digested with restriction enzyme NotI. The ends of the resulting fragment were blunted and then digested with NcoI. The plasmid pMON13773 (Figure 8) was then digested with restriction enzyme EcoRI, the ends blunted and then digested with NcoI. The resulting fragments were ligated resulting in plasmid pMON58044, which contained the AS gene under the control of the 7S promoter and NOS3' terminator (Figure 9).

Plasmid pMON58044 was then cut with restriction enzymes BglII and NcoI and ligated with a fragment that was generated by digesting pMON53084 (Figure 10) with BglII and NcoI. The resulting fragment was named pMON58045 (Figure 11) and contained the sequence for the *Arabidopsis* SSU1A transit peptide.

Finally, plasmid pMON58046 (Figure 12) was constructed by ligating the fragments generated by digesting pMON58045 (Figure 11) and pMON38207 (Figure 13) with restriction enzyme NotI. This resulted in the pMON58046 vector (Figure 12) that was used for soybean transformation.

Soybean Transformation By Microprojectile Bombardment

30 For the particle bombardment transformation method, commercially available soybean seeds (i.e., Asgrow A3244, A4922) were germinated overnight for approximately 18-24 hours and the meristem explants were excised. The primary leaves were removed to expose the meristems and the explants were

placed in targeting media with the meristems positioned perpendicular to the direction of the particle delivery.

The pMON58046 transformation vector described above was precipitated onto microscopic gold particles with CaCl_2 and spermidine and subsequently resuspended in ethanol. The suspension was coated onto a Mylar sheet that was then placed onto the electric discharge device. The particles were accelerated into the plant tissue by electric discharge at approximately 60% capacitance.

Following bombardment, the explants were placed in selection media (WPM + 0.075 mM glyphosate) (WPM = Woody Plant Medium (McCown & Lloyd, Proc. International Plant Propagation Soc., 30:421, 1981) minus BAP)) for 5-7 weeks to allow for selection and growth of transgenic shoots. Phenotype positive shoots were harvested approximately 5-7 weeks post-bombardment and placed into selective rooting media (BRM + 0.025mM glyphosate) (see below for BRM recipe) for 2-3 weeks. Shoots producing roots were transferred to the greenhouse and potted in soil. Shoots that remained healthy on selection, but did not produce roots were transferred to non-selective rooting media (BRM without glyphosate) for an additional two weeks. The roots from any shoots that produced roots off the selection were tested for expression of the plant selectable marker before transferring to the greenhouse and potting in soil. Plants were maintained under standard greenhouse conditions until R1 seed harvest.

The recipe used for Bean Rooting Medium (BRM) is provided below.

	<u>Compound</u>	<u>Quantity for 4L</u>
	MS Salts***	8.6g
25	Myo-inositol(cell culture grade)	0.40g
	SBRM Vitamin Stock**	8.0ml
	L-Cysteine (10mg/ml)	40.0ml
	Sucrose (ultra pure)	120g
	Adjust pH to 5.8	
30	Washed Agar	32g
	Additions after autoclaving:	
	SBRM/TSG Hormone Stock*	20.0ml

*SBRM/TSG Hormone Stock (per 1L of BRM): 3.0ml IAA (0.033mg/ml),
2.0ml sterile distilled water. Store stock in dark at 4 °C.

**SBRM Vitamin Stock (per 1L of stock): Glycine (1.0g), Nicotinic Acid
(0.25g), Pyridoxine HCl (0.25g), Thiamine HCl (0.25g).

- 5 ***3X Minor MS Salts (per 1L stock): H_2BO_3 (1.86g), $MnSO_4$ (5.07g),
 $ZnSO_4 \cdot H_2O$ (2.58g), KI (0.249g), 7.5 ul $NaMoO_4 \cdot 2H_2O$ (1.0mg/ml), 7.5 ul
 $CoSO_4 \cdot 5H_2O$ (1.0mg/ml), 7.5 ul $CoCl_2 \cdot 6H_2O$ (1.0mg/ml).
One ingredient at a time was added and dissolved, the volume was brought to
one liter with sterile distilled water, and the solution was stored in a foil-covered
10 bottle in the refrigerator for no longer than one month.

Soybean Transformation Using *Agrobacterium tumefaciens*

- For the *Agrobacterium* transformation method, commercially available
soybean seeds (Asgrow A3244, A4922) were germinated overnight
15 (approximately 10-12 hours) and the meristem explants were excised. The
primary leaves may or may not have been removed to expose the meristems and
the explants were placed in a wounding vessel.

- Agrobacterium* strain ABI containing the plasmid of interest was grown
to log phase. Cells were harvested by centrifugation and resuspended in
20 inoculation media containing inducers. Soybean explants and the induced
Agrobacterium culture were mixed no later than 14 hours from the time of
initiation of seed germination and wounded using sonication.

- Following wounding, explants were incubated in *Agrobacterium* for a
period of approximately one hour. Following this inoculation step, the
25 *Agrobacterium* was removed by pipetting and the explants were placed in co-
culture for 2-4 days. At this point, they were transferred to selection media
(WPM + 0.075 mM glyphosate + antibiotics to control *Agrobacterium*
overgrowth) for 5-7 weeks to allow selection and growth of transgenic shoots.

- Phenotype positive shoots were harvested approximately 5-7 weeks post-
30 bombardment and placed into selective rooting media (BRM + 0.025 mM
glyphosate) for 2-3 weeks. Shoots producing roots were transferred to the
greenhouse and potted in soil. Shoots that remained healthy on selection, but did
not produce roots were transferred to non-selective rooting media (BRM without

glyphosate) for an additional two weeks. The roots from any shoots that produced roots off the selection were tested for expression of the plant selectable marker glyphosate resistance before transferring to the greenhouse and potting in soil. Plants were maintained under standard greenhouse conditions until R1 seed
5 harvest.

Analysis of Amino Acid Content of R1 Seed

Mature R1 seed is produced and analyzed for free amino acid content using fluorescence detection as described in Agilent Technologies Technical
10 Bulletin REV14. Five seeds are chosen for single seed analysis from each event. Soy seeds expressing the AgroAS F298W or the AgroAS S51F mutant proteins generate very high amounts of tryptophan. Results are shown in Tables K and L.

Table K: Protein expression in Seeds Transformed with pMON58046

Pedigree	Trp average (ppm)	Protein present ?
Control	96	no
22817	9922	yes
22891	12955	yes
23026	7968	yes

5

Table L: AS Protein expression Correlated with pMON58123 Transformation

Pedigree	Trp average (ppm)	Protein present ?
Control	96	no
23562	88	no
23590	8795	yes
23911	388	no

10

AS Enzyme Activity in R1 Seed Transformed with Agro AS

Mature R1 seed is produced and analyzed for anthranilate synthase activity. Anthranilate synthase enzymatic activity was determined in R1 soy seeds carrying the AgroAS F298W (SEQ ID NO:65 or 91) or the Agro AS S51F (SEQ ID NO:60 or 86) mutant alleles. Very high levels of tryptophan-resistant anthranilate synthase activity was observed, consistent with the high amounts of tryptophan generated by these seeds. Results are shown in Tables M and N.

15

Table M: Specific activity of AS in R1 Seeds Transformed with pMON58046

20

Event	Seed number	Specific activity (pmoles/mg/min)	Specific activity (pmoles/mg/min) (+ 25 micromolar Trp)
Control		77.6	
23076	23076-1	100.5	1.04
	23076-2	4512.8	
	23076-3	9737.4	9290.4
	23076-4	136.12	
	23076-5	8992.5	9749.9

**Table N: Specific activity of AS in R1 Seeds
Transformed with pMON58123**

Event	Seed number	Specific activity (pmoles/mg/min)	Specific activity (pmoles/mg/min) (+ 25 micromolar Trp)
Control		83.7	32.7
23590	23590-1	891	692.3
	23590-2	466.2	186.5
	23590-3	71.7	38.3
	23590-4	320.5	316.2

5

EXAMPLE 7: Preparation of Transformation Vector Comprising *Ruta graveolens* Anthranilate Synthase α -Subunit

10 The anthranilate synthase α gene from *Ruta graveolens* (Genbank Accession No. GI 960291) provides another anthranilate synthase domain useful in the present invention (Bohlmann, J et al., Plant Phys 111 507-514 (1996)). One isoenzyme of anthranilate synthase present in the genome of *Ruta graveolens* demonstrates less susceptibility to feedback inhibition by L-tryptophan. This allele may also be useful in the present invention to elevate the levels of free L-tryptophan in transgenic plants. The vector pMON58030 (Figure 14) contains the *Ruta graveolens* anthranilate synthase α -subunit that is less sensitive to tryptophan inhibition. The *Ruta graveolens* anthranilate synthase α gene was PCR amplified from pMON58030 to provide a BamHI site at the 5' end and a BglII site at the 3' end of the *Ruta graveolens* anthranilate synthase α gene fragment by utilizing PCR primers that contained these two restriction enzyme sites:

5'-CAAAAGCTGGATCCCCACC-3' (SEQ ID NO:53) and

5'-CCTATCCGAGATCTCTCAACTCC-3' (SEQ ID NO:54).

25 The PCR fragment was purified, digested with the respective restriction enzymes, to form pMON58041, which contains the transcriptional fusion of the *Ruta graveolens* AS α to the napin promoter. The *Agrobacterium* mediated plant transformation plasmid, pMON58043, was created comprising the napin promoter, *Ruta graveolens* AS, NOS terminator, glyphosate resistance (CP4)

selectable marker and borders suitable for proper chromosomal integration of the cassette as described. The resulting plant transformation vector was used to transform plants using standard plant transformation techniques as described in Examples 2, 3 and 6.

5

EXAMPLE 8: Transforming multi-polypeptide anthranilate synthases into monomeric single polypeptide anthranilate synthases

Generation of a monomeric anthranilate synthase by fusion of selected multi-subunit enzymes is desirable, for example, to maximize the catalytic efficiency, to stabilize the enzyme, to achieve coordinated expression, for example, of subunits comprising activities of TrpE and TrpG and for effective communication between the two subunits. In some instances, it may be useful to employ TrpE or α -subunits from either plant or microbial source that are deregulated with respect to feedback inhibition by standard mutagenesis techniques or by rational design as described in the foregoing Examples, e.g. in Example 4. In other instances, wild type TrpE or α -subunits from either plant or microbial source are employed.

The C-terminus of the selected TrpE or α -subunit is linked to the N-terminus of the TrpG subunit or β -subunit, preferably with a peptide linker. A linker can be rationally designed to provide suitable spacing and flexibility for both subunits to properly align. Alternatively a linker can be identified by sequence alignment of monomeric and heterotetrameric anthranilate synthases. Examples of sequence alignments of monomeric and heterotetrameric anthranilate synthase forms are shown in Figures 21 and 35. It is also envisioned that it may be necessary to generate monomeric anthranilate synthases comprising heterologous subunit in order to maximize the benefits. For example, an α -subunit may be obtained from a bacterial source, for example, *E. coli* and fused to a β -subunit from a plant source, for example, *Arabidopsis*.

The novel protein produced can be introduced into plants, for example, as described in Examples 2, 3 or 6. The invention is not limited to the exact details shown and described, for it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention defined by the claims.

**EXAMPLE 9: Identification of anthranilate synthases
from genomic sequence databases.**

5 Monomeric anthranilate synthases as well as α and β domains useful in the invention can be identified by bioinformatics analysis by searching for example, genbank and/or swissprot databases using BLAST (www.ncbi.nlm.nih.gov/blast/). Useful query sequences to identify monomeric anthranilate synthase include, for example, domains of anthranilate synthase

10 such as the α -domain (GI 1004323) or β -domain (GI 1004324) from *Sulfolobus solfataricus*, or monomeric anthranilate synthase such as *Agrobacterium tumefaciens* AS (GI 15889565). Putative monomeric anthranilate synthase will have between 50% and 100% homology with the query sequence and should minimally contain 700 amino acids. If the AS- α -domain is used to query the

15 genomic database, in addition to identifying putative anthranilate synthase genes it is also likely to identify genes involved in PABA synthesis for example 4-amino-4-deoxychorismate (ADC) synthase. The monomeric ADC synthase genes can be easily identified away from putative monomeric AS genes based on the observation that the amidotransferase domain (β -domain) of ADC synthase

20 resides at the N-terminus of the protein whereas the amidotransferase domain (β -domain) of AS resides at the C-terminus. Monomeric anthranilate synthases useful in the present invention identified by bioinformatics analysis include, but are not limited to, for example, *Rhizobium meliloti* (GI 95177), *Mesorhizobium loti* (GI 13472468), *Brucella melitensis* (GI 17982357), *Nostoc sp.* PCC7120 (GI

25 17227910, GI 17230725), *Azospirillum brasilense* (GI 1174156), *Rhodopseudomonas palustris*, *Anabaena* M22983 (GI 152445). Figure 21 is an example of a sequence alignment of two monomeric anthranilate synthases (*Agrobacterium tumefaciens* and *Rhizobium meliloti*) with two heterotetrameric anthranilate synthases (*Sulfolobus solfataricus* and *Arabidopsis thaliana*) useful

30 in the present invention. Figure 35 is an example of a sequence alignment of several monomeric anthranilate synthases with the *Rhodopseudomonas palustris* heterotetrameric anthranilate synthase.

EXAMPLE 10: Optimized Codon Usage

This example sets forth a method of improving the expression of an anthranilate synthase gene in the seed of a plant by optimization of the codon usage.

- 5 The nucleotide sequence of the anthranilate synthase (AS) gene from wild type *Agrobacterium tumefaciens* (SEQ ID NO:1) was inspected for the presence of underexpressed codons. To identify underexpressed codons sequences of highly expressed seed proteins from corn and soybeans were examined for relative codon frequency. The relative codon usage frequencies are
- 10 shown in Table O represented in an expected value format. Expected value format can be exemplified as follows: Assume there are four codons that encode a given amino acid, and assume that they are used equally well, then each codon would be expected to account for 25% (0.25) of the frequency for that amino acid. However, due to redundancy, 0.25 was normalized to 1.0 to give a relative
- 15 score for each codon as compared to other codons that encode that amino acid. For this analysis, if a codon was more prevalent than the other choices for a given amino acid, it received a number that was greater than 1.0. Correspondingly, if a codon was less prevalent, it received a number less than 1.0. For this study, a particular codon was considered underrepresented if its relative codon usage
- 20 frequency was lower than 0.5.

- Using the results from Table O, a close examination of the wild type *Agrobacterium* AS sequence revealed that 125 codons were considered underrepresented (below the threshold of 0.5) in corn and soybeans (Table P). These underrepresented codons were replaced by more prevalent codons as
- 25 defined above. The modified nucleotide sequence is shown in Figure 36. Using bioinformatics tools, the resulting sequence was assembled and analyzed for integrity by translation and alignment of the nucleotide and protein sequences with the corresponding wild type AS sequences. While, the protein sequence was unchanged the nucleotide sequence of the optimized sequence had 94%
- 30 identity with the wild type *Agrobacterium* AS sequence (Figure 37). The optimized nucleotide sequence was analyzed for the absence of cryptic polyadenylation signals (AATAAA, AATAAT) and cryptic introns using Lasergene EditSeq (DNASTAR, Inc., Madison, WI) and Grail2 (Oak Ridge

National Laboratory, Oak Ridge, TN), respectively. No cryptic signals were found.

- The modified nucleotide sequence is synthesized using techniques well known in the art or by commercial providers such as Egea Biosciences, Inc. (San Diego, CA). The resulting nucleotide is cloned into an appropriate expression vector and tested for efficacy in corn, soybeans and *Arabidopsis* using procedures detailed in earlier examples of this specification.
- 5

**Table O: Relative codon usage frequencies
in maize and soybean seed-expressed genes¹.**

Codon	AA	Maize Seed	Soy Seed	Codon	AA	Maize Seed	Soy Seed
TTT	F	0.4211	0.7348	ATC	I	1.7143	1.0563
TTC	F	1.5789	1.2652	ATA	I	0.3673	0.6554
TTA	L	0.4557	0.3875	ATG	M	1.0000	1.0000
TTG	L	0.9494	1.2060	ACT	T	0.6153	1.0008
TCT	S	0.9624	1.4851	ACC	T	1.2213	2.1020
TCC	S	1.3707	1.1249	ACA	T	0.8372	0.7146
TCA	S	0.9107	1.0044	ACG	T	1.3262	0.1826
TCG	S	0.7851	0.3266	AAT	N	0.2885	0.5409
TAT	Y	0.2455	0.8861	AAC	N	1.7115	1.4591
TAC	Y	1.7545	1.3139	AAA	K	0.5333	0.9030
TGT	C	0.2778	0.7572	AAG	K	1.4667	1.0970
TGC	C	1.7222	1.2428	AGT	S	0.2679	0.9714
TGG	W	1.0000	1.0000	AGC	S	1.7032	1.0876
CTT	L	0.7975	1.6298	AGA	R	0.3913	1.9459
CTC	L	1.0610	1.6301	AGG	R	2.9185	1.3087
CTA	L	0.8544	0.5905	GTT	V	0.5714	1.2381
CTG	L	1.8820	0.5562	GTC	V	1.0119	0.6864
CCT	P	0.6500	1.5822	GTA	V	0.3810	0.3472
CCC	P	0.8520	0.7694	GTG	V	2.0357	1.7284
CCA	P	1.2240	1.5838	GCT	A	0.9876	1.3583
CCG	P	1.2740	0.0645	GCC	A	1.1618	1.1283
CAT	H	0.8438	0.6066	GCA	A	0.8011	1.2898
CAC	H	1.1563	1.3934	GCG	A	1.0495	0.2235
CAA	Q	0.8639	1.2162	GAT	D	0.8500	0.9523
CAG	Q	1.1361	0.7838	GAC	D	1.1500	1.0477
CGT	R	0.2582	0.5903	GAA	E	0.6818	1.0463
CGC	R	1.0082	1.1159	GAG	E	1.3182	0.9537
CGA	R	0.1957	0.6700	GGT	G	1.1268	1.1431
CGS	R	1.2283	0.3692	GGC	G	1.8758	0.8577
ATT	I	0.9184	1.2783	GGA	G	0.3085	1.2759
ATC	I	1.7143	1.0563	GGG	G	0.6889	0.9233

- 5 ¹ The relative codon frequencies are represented in the expected value format. This means that if there are four codons that encode a given amino acid, and they are used equally well, each codon is expected to account for 25% (0.25). Due to the redundancy, 0.25 was normalized to 1 to give a relative score for each codon as compared to all codons that encode that amino acid. In real life if a codon is more prevalent than the other choices for a given amino acid, it would get a number >1. And if it is less preferred than the other codons for the amino acid, it would get a number <1.
- 10

Table P. Underrepresented Agro AS codons and modifications for improved seed expression².

Codon	Codon (wt)	Amino Acid	Underrep in Crop ²	Codon	Codon (wt)	Amino Acid	Underrep in Crop	Codon	Codon (wt)	Amino Acid	Modified Underrep in Crop
2	GTA	V	GTG	177	TCG	S	TCC	481	GCG	A	GCC
3	ACG	T	ACC	179	GCG	A	GCC	485	AAT	N	AAC
9	GGA	G	GGT	180	CGT	R	CGC	489	CCG	P	CCA
10	GCG	A	GCC	181	CCG	P	CCA	504	ATA	I	ATC
15	ACG	T	ACC	185	CGT	R	CGC	508	CGT	R	CGC
16	AAA	K	AAG	190	TTT	F	TTC	520	CGT	R	CGC
21	GTC	V	GTG	201	TAT	Y	TAC	543	ACG	T	ACC
23	GGA	R	CGC	209	CGT	R	CGC	545	GCG	A	GCC
26	CGG	R	CGC	218	ACG	T	ACC	546	AAT	N	AAC
30	TAT	Y	TAC	219	ACG	T	ACC	547	TAT	Y	TAC
36	AAT	N	AAC	238	CCG	P	CCA	551	ACG	T	ACC
46	GCG	G	GGT	244	CGT	R	CGC	553	GCG	A	GCC
47	GCG	A	GCC	248	TAT	Y	TAC	554	ACG	T	ACC
48	GTT	V	GTG	276	CGT	R	CGC	556	TCG	S	TCC
49	TTT	F	TTC	280	AAT	N	AAC	559	AGA	R	AGG
50	TCG	S	TCC	281	CCG	P	CCA	561	CCG	P	CCA
53	TAT	Y	TAC	282	TCG	S	TCC	572	CCG	P	CCA
55	TAT	Y	TAC	283	GCG	A	GCC	578	TCG	S	TCC
56	CCG	P	CCA	290	GCG	A	GCC	580	GGA	G	GGT
58	CGT	R	CGC	293	CCG	P	CCA	584	CCG	P	CCA

Codon	Codon	Amino	Modified	Underrep	Codon	Codon	Amino	Modified	Underrep	Codon	Codon	Amino	Modified	Underrep
(wt)	Acid	Codon	In Crop	(wt)	Acid	Codon	In Crop	(wt)	Acid	Codon	In Crop	(wt)	Acid	Codon
64	ACG	T	ACC	soy	294	TCG	S	585	T	ACC	soy	585	T	ACC
69	CCG	P	CCA	soy	296	TAT	Y	592	ACG	T	ACC	592	ACG	T
70	CCG	P	CCA	soy	301	AAT	N	602	CCG	P	CCA	602	CCG	P
75	TGT	C	TGC	corn	307	TAT	Y	617	TAT	Y	TAC	617	TAT	Y
76	TTT	F	TTC	corn	312	TCG	S	633	TCG	S	TCC	633	TCG	S
85	TAT	Y	TAC	corn	313	CCG	P	652	ACG	T	ACC	652	ACG	T
86	AAT	N	AAC	corn, soy	322	CGT	R	655	CGT	R	CGC	655	CGT	R
97	ACG	T	ACC	soy	328	CCG	P	658	TCG	S	TCC	658	TCG	S
102	CCG	A	GCC	soy	329	ATA	I	667	CCG	P	CCA	667	CCG	P
112	TCG	S	TCC	soy	339	CCG	P	668	CGT	R	CGC	668	CGT	R
115	CGG	R	CGC	soy	352	TCG	S	680	ACG	T	ACC	680	ACG	T
123	CCG	P	CCA	soy	363	TCG	S	690	CCG	P	CCA	690	CCG	P
125	CGT	R	CGC	corn	376	CCG	P	698	CCG	P	CCA	698	CCG	P
133	TCG	S	TCC	soy	378	TCG	S	700	TCG	S	TCC	700	TCG	S
136	CCG	P	CCA	soy	390	TAT	Y	703	ACG	T	ACC	703	ACG	T
137	ACG	T	ACC	soy	411	TTT	F	705	GGA	G	GGT	705	GGA	G
143	AGA	R	AGG	corn	442	CCG	P	708	CCG	A	GCC	708	CCG	A
150	TAT	Y	TAC	corn	446	TAT	Y	711	CGG	R	CGC	711	CGG	R
151	TCG	S	TCC	soy	449	GCG	A	715	AAT	N	AAC	715	AAT	N
153	GCG	A	GCC	soy	460	AAT	N	724	GCG	A	GCC	724	GCG	A
155	TCG	S	TCC	soy	464	ACG	T	729	GCG	A	GCC	729	GCG	A
173	GCG	A	GCC	soy	469	GCG	R							

² The columns titled "Underrep in Crop" indicate in which crop (maize or soybean) a particular codon is underrepresented.

All publications and patents are incorporated by reference herein, as though individually incorporated by reference. The invention is not limited to the exact details shown and described, for it should be understood that many
5 variations and modifications may be made while remaining within the spirit and scope of the invention defined by the claims.

WHAT IS CLAIMED:

1. An isolated DNA encoding a monomeric anthranilate synthase, wherein the monomeric anthranilate synthase comprises a single polypeptide comprising an anthranilate synthase α -domain and an anthranilate synthase β -domain, and wherein
5 the monomeric anthranilate synthase is expressed in a plant.
2. The isolated DNA of claim 1, wherein expression of the monomeric anthranilate synthase elevates the level of L-tryptophan in the plant relative to an untransformed plant having the same genetic background.
- 10 3. The isolated DNA of claim 1, wherein the monomeric anthranilate synthase is an *Agrobacterium tumefaciens*, *Rhizobium meliloti*, *Mesorhizobium loti*, *Brucella melitensis*, *Nostoc sp.* PCC7120, *Azospirillum brasilense* or *Anabaena M22983* anthranilate synthase.
- 15 4. The isolated DNA of claim 1, wherein the monomeric anthranilate synthase comprises any one of SEQ ID NO:4, 7, 43, 58, 59, 60, 61, 62, 63, 64, 65, 69, 70, 77, 78, 79, 80, 81 or 82.
- 20 5. The isolated DNA of claim 1, wherein the isolated DNA comprises any one of SEQ ID NO:1, 75, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92 or 93.
- 25 6. The isolated DNA of claim 1, wherein the isolated DNA encodes a chimeric monomeric anthranilate synthase comprising a fusion of an anthranilate synthase α domain from one species and an anthranilate synthase β domain from a second species.

7. The isolated DNA of claim 1, wherein DNA encoding the α domain or the β domain is obtained from *Agrobacterium tumefaciens*, *Anabaena* M22983, *Arabidopsis thaliana*, *Azospirillum brasilense*, *Brucella melitensis*, *Escherichia coli*, *Euglena gracilis*, *Mesorhizobium loti*, *Nostoc sp.* PCC7120, *Rhizobium meliloti*, *Ruta graveolens*, *Rhodopseudomonas palustris*, *Salmonella typhimurium*, *Serratia marcescens*, *Sulfolobus solfataricus*, cotton, rice, wheat, tobacco or *Zea mays*.
8. The isolated DNA of claim 1, wherein the α domain or the β domain is at least a portion of any one of amino acid sequences SEQ ID NO:4, 5, 6, 7, 8, 43, 44, 45, 58, 59, 60, 61, 62, 63, 64, 65, 66, 69, 70, 77, 78, 79, 80, 81, 82, 99, 100, 101, 102 or 103.
9. The isolated DNA of claim 1, wherein the anthranilate synthase comprises a mutation that increases anthranilate synthase activity or reduces the sensitivity of the anthranilate synthase to inhibition by tryptophan or an analog thereof.
10. The isolated DNA of claim 9, wherein the mutation is in a tryptophan-binding pocket.
11. The isolated DNA of claim 9, wherein the mutation is within amino acid positions 25-60 or 200-225 or 290-300 or 370-375 when the anthranilate synthase amino acid sequence is aligned with a monomeric *Agrobacterium tumefaciens* anthranilate synthase having SEQ ID NO:4.
12. The isolated DNA of claim 9, wherein the mutation is:
- (a) at about position 48, replace Val with Phe;
 - (b) at about position 48, replace Val with Tyr;

- (c) at about position 51, replace Ser with Phe;
- (d) at about position 51, replace Ser with Cys;
- (e) at about position 52, replace Asn with Phe;
- (f) at about position 293, replace Pro with Ala;
- (g) at about position 293, replace Pro with Gly; or
- (h) at about position 298, replace Phe with Trp; and

wherein the position of the mutation is determined by alignment of the amino acid sequence of the anthranilate synthase with an *Agrobacterium tumefaciens* anthranilate synthase amino acid sequence.

13. The isolated DNA of claim 9, wherein the anthranilate synthase comprises any one of SEQ ID NO:58-65, 69 or 70.

14. The isolated DNA of claim 12, wherein the *Agrobacterium tumefaciens* anthranilate synthase amino acid sequence is SEQ ID NO:4.

15. The isolated DNA of claim 1, wherein the isolated DNA further encodes a plastid transit peptide.

16. The isolated DNA of claim 15, wherein the plastid transit peptide comprises SEQ ID NO:72 or 74.

17. The isolated DNA of claim 1, wherein the isolated DNA further encodes a selectable marker gene or a reporter gene.

18. The isolated DNA of claim 17, wherein the selectable marker gene, when expressed in a plant, imparts herbicide resistance to cells of said plant.

19. The isolated DNA of claim 18, wherein the herbicide resistance comprises resistance to glyphosate, glufosinate or dalapon.
20. The isolated DNA of claim 1, wherein the isolated DNA further encodes a *Bacillus thuringiensis* protein that, when expressed in a plant, imparts insect resistance to the
5 plant.
21. The isolated DNA of claim 1, wherein the plant is a dicot.
22. The isolated DNA of claim 21, wherein the plant is soybean or canola.
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23. The isolated DNA of claim 1, wherein the plant is a monocot.
24. The isolated DNA of claim 23, wherein the plant is maize, rice, wheat, barley or
15 sorghum.
25. The isolated DNA of claim 1, wherein the isolated DNA encoding the anthranilate synthase comprises a promoter operably linked thereto.
26. A vector comprising the isolated DNA of any one of claims 1- 25.
20
27. A seed comprising the isolated DNA of any one of claims 1- 25.
28. A transgenic plant comprising an isolated DNA encoding a monomeric anthranilate
25 synthase operably linked to a promoter, wherein the monomeric anthranilate synthase comprises an anthranilate synthase α domain and an anthranilate synthase β domain, and wherein the monomeric anthranilate synthase is expressed in the plant.

29. The transgenic plant of claim 28, wherein expression of the monomeric anthranilate synthase elevates the level of L-tryptophan in the plant relative to an untransformed plant having the same genetic background.

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30. The transgenic plant of claim 28, wherein the monomeric anthranilate synthase is an *Agrobacterium tumefaciens*, *Rhizobium meliloti*, *Mesorhizobium loti*, *Brucella melitensis*, *Nostoc sp.* PCC7120, *Azospirillum brasilense* or *Anabaena M22983* anthranilate synthase.

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31. The transgenic plant of claim 30, wherein the monomeric anthranilate synthase is a *Rhizobium meliloti* (Genbank Accession No. GI 95177), *Mesorhizobium loti* (Genbank Accession No. GI 13472468), *Brucella melitensis* (Genbank Accession No. GI 17982357), *Nostoc sp.* PCC7120 (Genbank Accession No. GI 17227910, GI 17230725), *Azospirillum brasilense* (Genbank Accession No. GI 1174156) or *Anabaena M22983* (Genbank Accession No. GI 152445) anthranilate synthase.

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32. The transgenic plant of claim 28 wherein the monomeric anthranilate synthase is a chimeric monomeric anthranilate synthase comprising a fusion of an anthranilate synthase α domain from one species linked to an anthranilate synthase β domain from a second species.

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33. The transgenic plant of claim 28, wherein DNA encoding the α domain or the β domain is obtained from *Agrobacterium tumefaciens*, *Anabaena M22983*, *Arabidopsis thaliana*, *Azospirillum brasilense*, *Brucella melitensis*, *Escherichia coli*, *Euglena gracilis*, *Mesorhizobium loti*, *Nostoc sp.* PCC7120, *Rhizobium meliloti*, *Ruta graveolens*, *Rhodopseudomonas palustris*, *Salmonella typhimurium*,

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Serratia marcescens, *Sulfolobus solfataricus*, soybean, rice, cotton, wheat, tobacco or *Zea mays*.

- 5 34. The transgenic plant of claim 28, wherein the α domain or the β domain is at least a portion of any one of amino acid sequences SEQ ID NO:4, 5, 6, 7, 8, 43, 44, 45, 58, 59, 60, 61, 62, 63, 64, 65, 66, 69, 70, 77, 78, 79 80, 81, 82, 99, 100, 101, 102 or 103.
- 10 35. The transgenic plant of claim 28, wherein the anthranilate synthase comprises a mutation that increases anthranilate synthase activity or reduces the sensitivity of the anthranilate synthase to inhibition by tryptophan or an analog thereof.
- 15 36. The transgenic plant of claim 35, wherein the mutation is in a tryptophan-binding pocket.
37. The transgenic plant of claim 35, wherein the mutation is within amino acid positions 25-60 or 200-225 or 290-300 or 370-375 when the anthranilate synthase amino acid sequence is aligned with a monomeric *Agrobacterium tumefaciens* anthranilate synthase having SEQ ID NO:4.
- 20 38. The transgenic plant of claim 35, wherein the mutation is:
- (a) at about position 48, replace Val with Phe;
 - (b) at about position 48, replace Val with Tyr;
 - (c) at about position 51, replace Ser with Phe;

25 (d) at about position 51, replace Ser with Cys;

 - (e) at about position 52, replace Asn with Phe;
 - (f) at about position 293, replace Pro with Ala;
 - (g) at about position 293, replace Pro with Gly; or

(h) at about position 298, replace Phe with Trp; and
wherein the position of the mutation is determined by alignment of the amino acid
sequence of the anthranilate synthase with an *Agrobacterium tumefaciens*
anthranilate synthase amino acid sequence.

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39. The transgenic plant of claim 35, wherein the anthranilate synthase comprises any
one of SEQ ID NO:58-65, 69 or 70.

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40. The transgenic plant of claim 38, wherein the *Agrobacterium tumefaciens*
anthranilate synthase amino acid sequence is SEQ ID NO:4.

41. The transgenic plant of claim 28, wherein the isolated DNA further comprises a
plastid transit peptide.

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42. The transgenic plant of claim 41, wherein the plastid transit peptide comprises SEQ
ID NO:72 or 74.

43. The transgenic plant of claim 28, wherein the isolated DNA further encodes a
selectable marker gene or a reporter gene.

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44. The transgenic plant of claim 43, wherein the selectable marker gene, when
expressed in a plant, imparts herbicide resistance to cells of said plant.

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45. The transgenic plant of claim 44, wherein the herbicide resistance comprises
resistance to glyphosate, glufosinate or dalapon.

46. The transgenic plant of claim 28, wherein the isolated DNA further encodes a *Bacillus thuringiensis* protein that, when expressed in a plant, imparts insect resistance to the plant.
- 5 47. The transgenic plant of claim 28, wherein the plant is a dicot.
48. The transgenic plant of claim 47, wherein the plant is soybean or canola.
49. The transgenic plant of claim 28, wherein the plant is a monocot.
- 10 50. The transgenic plant of claim 49, wherein the plant is maize, rice, wheat, barley or sorghum.
51. A seed of the transgenic plant of claim 28.
- 15 52. A transgenic plant comprising, operably linked to a promoter, an isolated DNA encoding an *Agrobacterium tumefaciens* anthranilate synthase, or a domain thereof.
53. The transgenic plant of claim 52, wherein the *Agrobacterium tumefaciens* anthranilate synthase, or domain thereof, is expressed so as to elevate the level of L-tryptophan in said plant.
- 20 54. The transgenic plant of claim 52, wherein the *Agrobacterium tumefaciens* anthranilate synthase comprises SEQ ID NO:4, 58, 59, 60, 61, 62, 63, 64, 65, 69 or 70.
- 25 55. The transgenic plant of claim 52, wherein the isolated DNA comprises SEQ ID NO:1, 75, 84, 85, 86, 87, 88, 89, 90, 91, 92 or 93.

56. A transgenic plant comprising, operably linked to a promoter, an isolated DNA encoding a chimeric monomeric anthranilate synthase, wherein the anthranilate synthase is a fusion of an anthranilate synthase α domain from one species and an anthranilate synthase β domain from a second species.
57. The transgenic plant of claim 56, wherein the chimeric monomeric anthranilate synthase is expressed so as to elevate the level of L-tryptophan in said plant.
58. The transgenic plant of claim 56, wherein DNA encoding the α domain or the β domain is obtained from *Agrobacterium tumefaciens*, *Anabaena* M22983, *Arabidopsis thaliana*, *Azospirillum brasilense*, *Brucella melitensis*, *Escherichia coli*, *Euglena gracilis*, *Mesorhizobium loti*, *Nostoc sp.* PCC7120, *Rhizobium meliloti*, *Ruta graveolens*, *Rhodopseudomonas palustris*, *Salmonella typhimurium*, *Serratia marcescens*, *Sulfolobus solfataricus*, soybean, rice, cotton, wheat, tobacco or *Zea mays*.
59. The transgenic plant of claim 56, wherein the α domain or the β domain is at least a portion of any one of amino acid sequences SEQ ID NO:4, 5, 6, 7, 8, 43, 44, 45, 58, 59, 60, 61, 62, 63, 64, 65, 66, 69, 70, 77, 78, 79, 80, 81, 82, 99, 100, 101, 102 or 103.
60. The transgenic plant of claim 52 or 56, wherein the anthranilate synthase comprises a mutation that increases anthranilate synthase activity or reduces the sensitivity of the anthranilate synthase to inhibition by tryptophan or an analog thereof.
61. The transgenic plant of claim 60, wherein the mutation is within amino acid positions 25-60 or 200-225 or 290-300 or 370-375 when the anthranilate synthase

amino acid sequence is aligned with a monomeric *Agrobacterium tumefaciens* anthranilate synthase having SEQ ID NO:4.

5 62. The transgenic plant of claim 60, wherein the mutation is in the tryptophan-binding pocket.

63. The transgenic plant of claim 60, wherein the mutation is:

- (a) at about position 48, replace Val with Phe;
- (b) at about position 48, replace Val with Tyr;
- 10 (c) at about position 51, replace Ser with Phe;
- (d) at about position 51, replace Ser with Cys;
- (e) at about position 52, replace Asn with Phe;
- (f) at about position 293, replace Pro with Ala;
- (g) at about position 293, replace Pro with Gly; or
- 15 (h) at about position 298, replace Phe with Trp; and

wherein the position of the mutation is determined by alignment of the amino acid sequence of the anthranilate synthase with an *Agrobacterium tumefaciens* anthranilate synthase amino acid sequence.

20 64. The transgenic plant of claim 60, wherein the anthranilate synthase comprises any one of SEQ ID NO:58-65, 69 or 70.

65. The transgenic plant of claim 63, wherein the *Agrobacterium tumefaciens* anthranilate synthase amino acid sequence is SEQ ID NO:4.

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66. The transgenic plant of claim 52 or 56, wherein the isolated DNA further comprises a plastid transit peptide.

67. The transgenic plant of claim 66, wherein the plastid transit peptide comprises SEQ ID NO:72 or 74.
- 5 68. The transgenic plant of claim 52 or 56, wherein the isolated DNA further encodes a selectable marker gene or a reporter gene.
69. The transgenic plant of claim 68, wherein the selectable marker gene, when expressed in a plant, imparts herbicide resistance to cells of said plant.
- 10 70. The transgenic plant of claim 69, wherein the herbicide resistance comprises resistance to glyphosate, glufosinate or dalapon.
71. The transgenic plant of claim 52 or 56, wherein the isolated DNA further encodes a *Bacillus thuringiensis* protein that, when expressed in a plant, imparts insect resistance to the plant.
- 15 72. The transgenic plant of claim 52 or 56, wherein the plant is a dicot.
73. The transgenic plant of claim 72, wherein the plant is soybean or canola.
- 20 74. The transgenic plant of claim 52 or 56, wherein the plant is a monocot.
75. The transgenic plant of claim 72, wherein the plant is maize, rice, wheat, barley or sorghum.
- 25 76. A seed of the transgenic plant of claim 52 or 56.

77. A transgenic plant comprising an isolated DNA encoding an α domain of anthranilate synthase from *Zea mays* that comprises SEQ ID NO:5 or SEQ ID NO:66 operably linked to a promoter.
- 5 78. The transgenic plant of claim 77, wherein the isolated DNA comprises SEQ ID NO:2, SEQ ID NO:67 or SEQ ID NO:68 operably linked to a promoter.
79. The transgenic plant of claim 77, wherein the α domain of monomeric anthranilate synthase is expressed so as to elevate the level of L-tryptophan in said plant.
- 10 80. The transgenic plant of claim 77, wherein the domain has at least one mutation that increases anthranilate synthase activity or reduces the sensitivity of the domain to inhibition by tryptophan or an analog thereof.
- 15 81. The transgenic plant of claim 77, wherein the mutation is in a tryptophan-binding pocket.
82. The transgenic plant of claim 77, wherein the isolated DNA further encodes a plastid transit peptide.
- 20 83. The transgenic plant of claim 82, wherein the plastid transit peptide comprises SEQ ID NO:72 or 74.
84. The transgenic plant of claim 77, wherein the isolated DNA further encodes a selectable marker gene or a reporter gene.
- 25 85. The transgenic plant of claim 84, wherein the selectable marker gene, when expressed in a plant, imparts herbicide resistance to cells of said plant.

86. The transgenic plant of claim 85, wherein the herbicide resistance comprises resistance to glyphosate, glufosinate or dalapon.
- 5 87. The transgenic plant of claim 77, wherein the isolated DNA further encodes a *Bacillus thuringiensis* protein that, when expressed in a plant, imparts insect resistance to the plant.
88. The transgenic plant of claim 77, wherein the plant is a dicot.
- 10 89. The transgenic plant of claim 88, wherein the plant is soybean or canola.
90. The transgenic plant of claim 77, wherein the plant is a monocot.
- 15 91. The transgenic plant of claim 90, wherein the plant is maize, rice, wheat, barley or sorghum.
92. A seed of the transgenic plant of claim 77.
- 20 93. A method for altering the tryptophan content in a plant comprising:
- (b) introducing into regenerable cells of a plant a transgene comprising an isolated DNA encoding a monomeric anthranilate synthase comprising an anthranilate synthase α domain and an anthranilate synthase β domain, wherein the isolated DNA is operably linked to a promoter functional in a
- 25 plant cell, to yield transformed plant cells; and
- (c) regenerating a plant from said transformed plant cells wherein the cells of the plant express the monomeric anthranilate synthase encoded by the isolated DNA in an amount effective to increase the tryptophan content in

the plant relative to the tryptophan content in an untransformed plant of the same genetic background.

- 5 94. The method of claim 93, wherein the monomeric anthranilate synthase is an *Agrobacterium tumefaciens*, *Rhizobium meliloti*, *Mesorhizobium loti*, *Brucella melitensis*, *Nostoc sp.* PCC7120, *Azospirillum brasilense* or *Anabaena M22983* anthranilate synthase.
- 10 95. The method of claim 93, wherein the monomeric anthranilate synthase comprises any one of SEQ ID NO:4, 7, 43, 58, 59, 60, 61, 62, 63, 64, 65, 69, 70, 77, 78, 79, 80, 81 or 82.
- 15 96. The method of claim 93, wherein the isolated DNA comprises any one of SEQ ID NO:1, 75, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92 or 93.
97. The method of claim 93, wherein the isolated DNA encodes a chimeric monomeric anthranilate synthase comprising a fusion of an anthranilate synthase α domain from one species and an anthranilate synthase β domain from a second species.
- 20 98. The method of claim 93 wherein DNA encoding the α domain or the β domain is obtained from *Agrobacterium tumefaciens*, *Anabaena M22983*, *Arabidopsis thaliana*, *Azospirillum brasilense*, *Brucella melitensis*, *Escherichia coli*, *Euglena gracilis*, *Mesorhizobium loti*, *Nostoc sp.* PCC7120, *Rhizobium meliloti*, *Ruta graveolens*, *Rhodopseudomonas palustris*, *Salmonella typhimurium*, *Serratia marcescens*, *Sulfolobus solfataricus*, soybean, rice, cotton, wheat, tobacco or *Zea mays*.
- 25

99. The method of claim 97, wherein the α domain or the β domain is at least a portion of any one of amino acid sequences SEQ ID NO:4, 5, 6, 7, 8, 43, 44, 45, 58, 59, 60, 61, 62, 63, 64, 65, 66, 69, 70, 77, 78, 79 80, 81, 82, 99, 100, 101, 102 or 103.
- 5 100. The method of claim 93, wherein the isolated DNA further encodes a plastid transit peptide.
101. The method of claim 100, wherein the plastid transit peptide comprises SEQ ID NO:72 or 74.
- 10 102. The method of claim 93, wherein the isolated DNA further encodes a selectable marker gene or a reporter gene.
103. The method of claim 102, wherein the selectable marker gene, when expressed in a plant, imparts herbicide resistance to cells of said plant.
- 15 104. The method of claim 103, wherein the herbicide resistance comprises resistance to glyphosate, glufosinate or dalapon.
- 20 105. The method of claim 93, wherein the isolated DNA further encodes a *Bacillus thuringiensis* protein that, when expressed in a plant, imparts insect resistance to the plant.
- 25 106. The method of claim 93, wherein the anthranilate synthase comprises a mutation that increases anthranilate synthase activity or that reduces the sensitivity of the anthranilate synthase to inhibition by tryptophan or an analog thereof.

107. The method of claim 106, wherein the mutation is within amino acid positions 25-60 or 200-225 or 290-300 or 370-375 when the anthranilate synthase amino acid sequence is aligned with a monomeric *Agrobacterium tumefaciens* anthranilate synthase having SEQ ID NO:4.
- 5 108. The method of claim 106, wherein the mutation is in the tryptophan-binding pocket.
109. The method of claim 106, wherein the mutation is:
- 10 (a) at about position 48, replace Val with Phe;
(b) at about position 48, replace Val with Tyr;
(c) at about position 51, replace Ser with Phe;
(d) at about position 51, replace Ser with Cys;
(e) at about position 52, replace Asn with Phe;
- 15 (f) at about position 293, replace Pro with Ala;
(g) at about position 293, replace Pro with Gly; or
(h) at about position 298, replace Phe with Trp; and
wherein the position of the mutation is determined by alignment of the amino acid sequence of the anthranilate synthase with an *Agrobacterium tumefaciens* anthranilate synthase amino acid sequence.
- 20 110. The method of claim 109 wherein the anthranilate synthase comprises any one of SEQ ID NO:58-65, 69 or 70.
- 25 111. The method of claim 109 wherein the *Agrobacterium tumefaciens* anthranilate synthase amino acid sequence is SEQ ID NO:4.
112. The method of claim 93, wherein the plant is a dicot.

113. The method of claim 112, wherein the plant is soybean or canola.
114. The method of claim 93, wherein the plant is a monocot.
- 5 115. The method of claim 114 wherein the plant is maize, rice, wheat, barley or sorghum.
116. A method for altering the tryptophan content in a plant comprising:
- 10 (a) introducing into regenerable cells of a plant a transgene comprising an isolated DNA encoding an α domain of anthranilate synthase from *Zea mays* that comprises SEQ ID NO:5 or SEQ ID NO:66, operably linked to a promoter functional in a plant cell and to yield transformed plant cells; and
- 15 (b) regenerating a plant from said transformed plant cells wherein the cells of the plant express the anthranilate synthase encoded by the isolated DNA in an amount effective to increase the tryptophan content in the plant relative to the tryptophan content in an untransformed plant of the same genetic background.
- 20 117. The method of claim 116, wherein the α domain of anthranilate synthase has a mutation that increases anthranilate synthase activity or reduces the sensitivity of the domain to inhibition by tryptophan or an analog thereof.
- 25 118. The method of claim 116 wherein the mutation is in a tryptophan-binding pocket.
119. The method of claim 116, wherein the plant is a dicot.

120. The method of claim 119, wherein the plant is soybean or canola.
121. The method of claim 116, wherein the plant is a monocot.
- 5 122. The method of claim 121, wherein the plant is maize, rice, wheat, barley or sorghum.
123. The method of claim 116, wherein the isolated DNA further encodes a selectable marker gene or a reporter gene.
- 10 124. The method of claim 123, wherein the selectable marker gene, when expressed in a plant, imparts herbicide resistance to cells of said plant.
125. The method of claim 124, wherein the herbicide resistance comprises resistance to glyphosate, glufosinate or dalapon.
- 15 126. The method of claim 116, wherein the isolated DNA further encodes a *Bacillus thuringiensis* protein that, when expressed in a plant, imparts insect resistance to the plant.
- 20 127. A method for making an animal feed or a human food comprising:
- (a) introducing into regenerable cells of a plant a transgene comprising an isolated DNA encoding a monomeric anthranilate synthase comprising an anthranilate synthase α domain and an anthranilate synthase β domain, wherein the isolated DNA is operably linked to a promoter functional in a plant cell, to yield transformed plant cells; and
- 25 (b) regenerating a plant from said transformed plant cells wherein the cells of the plant express the monomeric anthranilate synthase encoded by the

isolated DNA in an amount effective to increase the tryptophan content in the plant relative to the tryptophan content in an untransformed plant of the same genetic background.

- 5 128. The method of claim 127, wherein the monomeric anthranilate synthase is an *Agrobacterium tumefaciens*, *Rhizobium meliloti*, *Mesorhizobium loti*, *Brucella melitensis*, *Nostoc* sp. PCC7120, *Azospirillum brasilense* or *Anabaena* M22983 anthranilate synthase.
- 10 129. The method of claim 127, wherein the monomeric anthranilate synthase comprises any one of SEQ ID NO:4, 7, 43, 58, 59, 60, 61, 62, 63, 64, 65, 69, 70, 77, 78, 79, 80, 81 or 82.
- 15 130. The method of claim 127, wherein the isolated DNA comprises any one of SEQ ID NO:1, 75, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92 or 93.
- 20 131. The method of claim 127, wherein the isolated DNA encodes a chimeric monomeric anthranilate synthase comprising a fusion of an anthranilate synthase α domain from one species and an anthranilate synthase β domain from a second species.
- 25 132. The method of claim 127, wherein DNA encoding the α domain or the β domain is obtained from *Agrobacterium tumefaciens*, *Anabaena* M22983, *Arabidopsis thaliana*, *Azospirillum brasilense*, *Brucella melitensis*, *Escherichia coli*, *Euglena gracilis*, *Mesorhizobium loti*, *Nostoc* sp. PCC7120, *Rhizobium meliloti*, *Ruta graveolens*, *Rhodopseudomonas palustris*, *Salmonella typhimurium*, *Serratia marcescens*, *Sulfolobus solfataricus*, soybean, rice, cotton, wheat, tobacco or *Zea mays*.

133. The method of claim 127, wherein the α domain or the β domain is at least a portion of any one of amino acid sequences SEQ ID NO:4, 5, 6, 7, 8, 43, 44, 45, 58, 59, 60, 61, 62, 63, 64, 65, 66, 69, 70, 77, 78, 79 80, 81, 82, 99, 100, 101, 102 or 103.
134. The method of claim 127, wherein the anthranilate synthase comprises a mutation that increases anthranilate synthase activity or reduces the sensitivity of the anthranilate synthase to inhibition by tryptophan or an analog thereof.
135. The method of claim 134, wherein the mutation is in the tryptophan-binding pocket.
136. The method of claim 134, wherein the mutation is:
- (a) at about position 48, replace Val with Phe;
 - (b) at about position 48, replace Val with Tyr;
 - (c) at about position 51, replace Ser with Phe;
 - (d) at about position 51, replace Ser with Cys;
 - (e) at about position 52, replace Asn with Phe;
 - (f) at about position 293, replace Pro with Ala;
 - (g) at about position 293, replace Pro with Gly; or
 - (h) at about position 298, replace Phe with Trp; and
- wherein the position of the mutation is determined by alignment of the amino acid sequence of the anthranilate synthase with an *Agrobacterium tumefaciens* anthranilate synthase amino acid sequence.
137. The method of claim 136, wherein the *Agrobacterium tumefaciens* anthranilate synthase amino acid sequence comprises SEQ ID NO:4.

138. The method of claim 134, wherein the anthranilate synthase comprises any one of SEQ ID NO:58-65, 69 or 70.
- 5 139. The method of claim 127, wherein the isolated DNA further encodes a plastid transit peptide.
140. The method of claim 139, wherein the plastid transit peptide comprises SEQ ID NO:72 or 74.
- 10 141. The method of claim 127 wherein the isolated DNA further encodes a selectable marker gene or a reporter gene.
142. The method of claim 141, wherein the selectable marker gene, when expressed in a plant, imparts herbicide resistance to cells of said plant.
- 15 143. The method of claim 142, wherein the herbicide resistance comprises resistance to glyphosate, glufosinate or dalapon.
- 20 144. The method of claim 127, wherein the isolated DNA further encodes a *Bacillus thuringiensis* protein that, when expressed in a plant, imparts insect resistance to the plant.
145. The method of claim 127, wherein the plant is a dicot.
- 25 146. The method of claim 145 wherein the plant is soybean or canola.
147. The method of claim 127 wherein the plant is a monocot.

148. The method of claim 147 wherein the plant is maize, rice, wheat, barley or sorghum.
- 5 149. An animal feed or human food comprising at least a portion of a plant that comprises an isolated DNA encoding a monomeric anthranilate synthase comprising an anthranilate synthase α domain and a anthranilate synthase β domain, wherein the cells of the plant can express the monomeric anthranilate synthase encoded by the isolated DNA.
- 10 150. The animal feed or human food of claim 149, wherein the cells of the plant can express the monomeric anthranilate synthase in an amount effective to increase the tryptophan content in the plant relative to the tryptophan content in an untransformed plant of the same genetic background.
- 15 151. The animal feed or human food of claim 149, wherein the portion of the plant comprises a seed, a leaf, a stem, a root, a tuber, or a fruit.
- 20 152. The animal feed or human food of claim 149, wherein the monomeric anthranilate synthase is an *Agrobacterium tumefaciens*, *Rhizobium meliloti*, *Mesorhizobium loti*, *Brucella melitensis*, *Nostoc sp. PCC7120*, *Azospirillum brasilense* or *Anabaena M22983* anthranilate synthase.
- 25 153. The animal feed or human food of claim 149, wherein the monomeric anthranilate synthase comprises any one of SEQ ID NO:4, 7, 43, 58, 59, 60, 61, 62, 63, 64, 65, 69, 70, 77, 78, 79, 80, 81 or 82.

154. The animal feed or human food of claim 149, wherein the isolated DNA comprises any one of SEQ ID NO:1, 75, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92 or 93.
- 5 155. The animal feed or human food of claim 149, wherein the isolated DNA encodes a chimeric monomeric anthranilate synthase comprising a fusion of an anthranilate synthase α domain from one species and an anthranilate synthase β domain from a second species.
- 10 156. The animal feed or human food of claim 155, wherein DNA encoding the α domain or the β domain is obtained from *Agrobacterium tumefaciens*, *Anabaena* M22983, *Arabidopsis thaliana*, *Azospirillum brasilense*, *Brucella melitensis*, *Escherichia coli*, *Euglena gracilis*, *Mesorhizobium loti*, *Nostoc sp.* PCC7120, *Rhizobium meliloti*, *Ruta graveolens*, *Rhodopseudomonas palustris*, *Salmonella* typhimurium, *Serratia marcescens*, *Sulfolobus solfataricus*, soybean, rice, cotton, wheat, tobacco or *Zea mays*.
- 15 157. The animal feed or human food of claim 149, wherein the α domain or the β domain is at least a portion of any one of amino acid sequences SEQ ID NO:4, 5, 6, 7, 8, 43, 44, 45, 58, 59, 60, 61, 62, 63, 64, 65, 66, 69, 70, 77, 78, 79 80, 81, 82, 99, 100, 101, 102 or 103.
- 20 158. The animal feed or human food of claim 149, wherein the anthranilate synthase comprises a mutation that increases anthranilate synthase activity or reduces the sensitivity of the anthranilate synthase to inhibition by tryptophan or an analog thereof.
- 25

159. The animal feed or human food of claim 158, wherein the mutation is within amino acid positions 25-60 or 200-225 or 290-300 or 370-375 when the anthranilate synthase amino acid sequence is aligned with a monomeric *Agrobacterium tumefaciens* anthranilate synthase having SEQ ID NO:4.
- 5
160. The animal feed or human food of claim 158, wherein the mutation is in a tryptophan-binding pocket.
161. The animal feed or human food of claim 158, wherein the mutation is:
- 10
- (a) at about position 48, replace Val with Phe;
 - (b) at about position 48, replace Val with Tyr;
 - (c) at about position 51, replace Ser with Phe;
 - (d) at about position 51, replace Ser with Cys;
 - (e) at about position 52, replace Asn with Phe;
 - 15
 - (f) at about position 293, replace Pro with Ala;
 - (g) at about position 293, replace Pro with Gly; or
 - (h) at about position 298, replace Phe with Trp; and
- wherein the position of the mutation is determined by alignment of the amino acid sequence of the anthranilate synthase with an *Agrobacterium tumefaciens* anthranilate synthase amino acid sequence.
- 20
162. The animal feed or human food of claim 149, wherein the anthranilate synthase comprises any one of SEQ ID NO:58-65, 69 or 70.
- 25
163. The animal feed or human food of claim 161, wherein the *Agrobacterium tumefaciens* anthranilate synthase amino acid sequence is SEQ ID NO:4.

164. The animal feed or human food of claim 149, wherein the isolated DNA further encodes a plastid transit peptide.
- 5 165. The animal feed or human food of claim 164, wherein the plastid transit peptide comprises SEQ ID NO:72 or 74.
166. The animal feed or human food of claim 149, wherein the isolated DNA further encodes a selectable marker gene or a reporter gene.
- 10 167. The animal feed or human food of claim 166, wherein the selectable marker gene, when expressed in a plant, imparts herbicide resistance to cells of said plant.
168. The animal feed or human food of claim 167, wherein the herbicide resistance comprises resistance to glyphosate, glufosinate or dalapon.
- 15 169. The animal feed or human food of claim 149, wherein the isolated DNA further encodes a *Bacillus thuringiensis* protein that, when expressed in a plant, imparts insect resistance to the plant.
- 20 170. The animal feed or human food of claim 149, wherein the plant is a dicot.
171. The animal feed or human food of claim 170, wherein the plant is soybean or canola.
- 25 172. The animal feed or human food of claim 149, wherein the plant is a monocot.
173. The animal feed or human food of claim 172, wherein the plant is maize, rice, wheat, barley or sorghum.

174. An isolated DNA encoding an anthranilate synthase comprising a polypeptide having at least 90% sequence identity with SEQ ID NO:4.
- 5 175. An isolated DNA encoding an anthranilate synthase, comprising a DNA having at least 60% sequence identity with SEQ ID NO:1.
176. The isolated DNA of claim 174 or 175, wherein the isolated DNA comprises at least twenty nucleotides and that hybridizes to the complement of a DNA having
10 SEQ ID NO:1 under stringent hybridization conditions.
177. The isolated DNA of claim 176, wherein the stringent hybridization conditions comprise washing at 42°C in 0.2 x SSC.
- 15 178. The isolated DNA of claim 176, wherein the isolated DNA comprises any one of SEQ ID NO:9-42 or 46-56.
179. An isolated DNA encoding an α domain of anthranilate synthase from *Zea mays*, that comprises amino acid sequence SEQ ID NO:5 or SEQ ID NO:66.
- 20 180. An isolated DNA encoding an α domain of anthranilate synthase from *Zea mays* that comprises nucleotide sequence SEQ ID NO:2, SEQ ID NO:67 or SEQ ID NO:68.
- 25 181. The isolated DNA of claim 179 or 180, wherein the α domain of anthranilate synthase can be expressed in a plant so as to elevate the level of L-tryptophan in the plant.

182. The isolated DNA of claim 179 or 180, wherein the domain has at least one mutation that reduces the sensitivity of the domain to inhibition by tryptophan or an analog thereof.
- 5 183. The isolated DNA of claim 179 or 180, wherein the mutation is in a tryptophan-binding pocket.
184. The isolated DNA of claim 179 or 180, wherein the isolated DNA further encodes a selectable marker gene or a reporter gene.
- 10 185. The isolated DNA of claim 184, wherein the selectable marker gene, when expressed in a plant, imparts herbicide resistance to cells of a plant.
186. The isolated DNA of claim 185, wherein the herbicide resistance comprises resistance to glyphosate, glufosinate or dalapon.
- 15 187. The isolated DNA of claim 179 or 180, wherein the isolated DNA further encodes a *Bacillus thuringiensis* protein that, when expressed in a plant, imparts insect resistance to the plant.
- 20 188. The isolated DNA of claim 179 or 180, wherein the plant is a dicot.
189. The isolated DNA of claim 188, wherein the plant is soybean or canola.
- 25 190. The isolated DNA of claim 179 or 180, wherein the plant is a monocot.
191. The isolated DNA of claim 190, wherein the plant is maize, rice, wheat, barley or sorghum.

192. The isolated DNA of claim 179 or 180, wherein the isolated DNA encoding the anthranilate synthase comprises a promoter operably linked thereto.
- 5 193. A vector comprising the isolated DNA of claim 179 or 180.
194. An isolated DNA encoding a mutant anthranilate synthase, wherein the mutation comprises:
- (a) at about position 48, replace Val with Phe;
 - 10 (b) at about position 48, replace Val with Tyr;
 - (c) at about position 51, replace Ser with Phe;
 - (d) at about position 51, replace Ser with Cys;
 - (e) at about position 52, replace Asn with Phe;
 - (f) at about position 293, replace Pro with Ala;
 - 15 (g) at about position 293, replace Pro with Gly; or
 - (h) at about position 298, replace Phe with Trp; and
- wherein the position of the mutation is determined by alignment of the amino acid sequence of the anthranilate synthase with an *Agrobacterium tumefaciens* anthranilate synthase amino acid sequence.
- 20 195. The isolated DNA of claim 194, wherein the anthranilate synthase comprises any one of SEQ ID NO:58-65.
196. The isolated DNA of claim 194, wherein the *Agrobacterium tumefaciens*
- 25 anthranilate synthase amino acid sequence is SEQ ID NO:4.
197. A method for producing tryptophan comprising: culturing a prokaryotic or eukaryotic host cell comprising an isolated DNA under conditions sufficient to

express a monomeric anthranilate synthase encoded by the isolated DNA, wherein the monomeric anthranilate synthase comprises an anthranilate synthase α domain and a anthranilate synthase β domain, and wherein the conditions sufficient to express a monomeric anthranilate synthase comprise nutrients and precursors sufficient for the host cell to synthesize tryptophan utilizing the monomeric anthranilate synthase.

198. The method of claim 197, wherein the method further comprises producing a phenylpropanoid, a flavonoid, an isoflavonoid, an indole, an indole alkaloid, or an indole glucosinolate.

199. The method of claim 197, wherein the monomeric anthranilate synthase is an *Agrobacterium tumefaciens*, *Rhizobium meliloti*, *Mesorhizobium loti*, *Brucella melitensis*, *Nostoc sp.* PCC7120, *Azospirillum brasilense* or *Anabaena* M22983 anthranilate synthase.

200. The method of claim 197, wherein the monomeric anthranilate synthase comprises any one of SEQ ID NO:4, 7, 43, 58, 59, 60, 61, 62, 63, 64, 65, 69, 70, 77, 78, 79, 80, 81 or 82.

201. The method of claim 197, wherein the isolated DNA comprises any one of SEQ ID NO:1, 75, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92 or 93.

202. The method of claim 197, wherein the isolated DNA encodes a chimeric monomeric anthranilate synthase comprising a fusion of an anthranilate synthase α domain from one species and an anthranilate synthase β domain from a second species.

203. The method of claim 197, wherein DNA encoding the α domain or the β domain is obtained from *Agrobacterium tumefaciens*, *Anabaena* M22983, *Arabidopsis thaliana*, *Azospirillum brasilense*, *Brucella melitensis*, *Escherichia coli*, *Euglena gracilis*, *Mesorhizobium loti*, *Nostoc sp.* PCC7120, *Rhizobium meliloti*, *Ruta graveolens*, *Rhodopseudomonas palustris*, *Salmonella typhimurium*, *Serratia marcescens*, *Sulfolobus solfataricus*, soybean, rice, cotton, wheat, tobacco or *Zea mays*.
204. The method of claim 197, wherein the α domain or the β domain is at least a portion of any one of amino acid sequences SEQ ID NO:4, 5, 6, 7, 8, 43, 44, 45, 58, 59, 60, 61, 62, 63, 64, 65, 66, 69, 70, 77, 78, 79, 80, 81, 82, 99, 100, 101, 102 or 103.
205. The method of claim 197, wherein the anthranilate synthase comprises a mutation that increases anthranilate synthase activity or reduces the sensitivity of the anthranilate synthase to inhibition by tryptophan or an analog thereof.
206. The method of claim 205, wherein the mutation is within amino acid positions 25-60 or 200-225 or 290-300 or 370-375 when the anthranilate synthase amino acid sequence is aligned with a monomeric *Agrobacterium tumefaciens* anthranilate synthase having SEQ ID NO:4.
207. The method of claim 205, wherein the mutation is in a tryptophan-binding pocket.
208. The method of claim 205, wherein the mutation is:
- (a) at about position 48, replace Val with Phe;
 - (b) at about position 48, replace Val with Tyr;
 - (c) at about position 51, replace Ser with Phe;

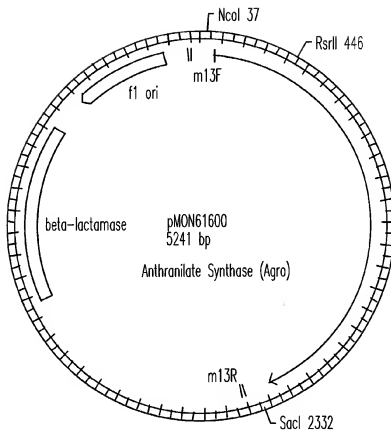
- (d) at about position 51, replace Ser with Cys;
- (e) at about position 52, replace Asn with Phe;
- (f) at about position 293, replace Pro with Ala;
- (g) at about position 293, replace Pro with Gly; or
- (h) at about position 298, replace Phe with Trp; and

wherein the position of the mutation is determined by alignment of the amino acid sequence of the anthranilate synthase with an *Agrobacterium tumefaciens* anthranilate synthase amino acid sequence.

209. The method of claim 197, wherein the anthranilate synthase comprises any one of SEQ ID NO:58-65, 69 or 70.

210. The method of claim 208, wherein the *Agrobacterium tumefaciens* anthranilate synthase amino acid sequence is SEQ ID NO:4.

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*Fig. 1*

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1  MVTIIQDDGAETYETKGGIQVSRKRPTDYANADINIDYIEKLDSHRGAVFS 50
  | : | : | | | | | | | | | | | | | | | | | | | | | | | | | |
1  MAAVILEDGAESYTTKGGIVVTRRRREASYSDAIAGYVDRDLERRGAVFS 50
  | : | : | | | | | | | | | | | | | | | | | | | | | | | | | |

51  SNYEYPGRYTRWDTAIVDPPLGISCFGRKMWIEAYNGRGEVLLDFITEKL 100
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
51  SNYEYPGRYTRWDTAVVDPPLAISSFGRLWIEAYNERGEVLLALIAEDL 100
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

101 KATPDLTLGASSTRRLDLTVNEPDRVFTTEERSKIPTVFTALRAIVDLFY 150
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
101 KSVADITLGSALAARRLDLTINEPDRVFTTEERSKMPTVFTVLRAVINLFH 150
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

151 SSADSAIGLFGAFGYDLAFQFDAIKLSLARPEDQORDMVLFLPDEILVVDH 200
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
151 SEEDSNLGLYGAFGYDLAFQFDAIELKLSRPDDQORDMVLFLPDEILVVDH 200
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

201 YSAKAWIDRYDFEKDGMTTDGKSSDITPDPFKTTDTTIPPKGDHRPGEYSE 250
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
201 YAAKAWIDRYDFARENLSLEGKAADIAPEPFRSVDSIPPHGDHRPGEYAE 250
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

251 LVVKAKESFRRGDLFEVVPQKFMERCESNPSAISRLKAINPSPYSFFI 300
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
251 LVVKAKESFRRGDLFEVVPQKFYERCESRPSEISNRLKAINPSPYSFFI 300
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

301 NLGDQEYLVGASPEMFVRVSGRRIETCPISGTIKRGDDPIADSEQILKLL 350
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
301 NLGQYLVGASPEMFVRVSGRRIETCPISGTIKRGDDPIADSEQILKLL 350
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

351 NSKKDESELTMCSDVDRNDKSRVCEPGSVKVGIRRRQIEMYSRLIHTVDHI 400
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
351 NSKKDESELTMCSDVDRNDKSRVCVPGSVKVGIRRRQIEMYSRLIHTVDHI 400
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

401 EGRLRDMDAFDGFLSHAWAVTVTGA PKLWAMRFIEGHEKSPRAWYGGAI 450
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
401 EGRLRDMDAFDGFLSHAWAVTVTGA PKLWAMRFIESHEKSPRAWYGGAI 450
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

451 GMVGFNGDMNTGLTLRTIRIKDGAIEVRAGATLLNDSNPQEEEEAETELKA 500
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
451 GMVGFNGDMNTGLTLRTIRIKDGAIEVRAGATLLYDSNPQEEEEAETELKA 500
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

501 SAMISAIRDAKGTNSAATKRDAKVGTVKILLVDHEDSFVHTLANYFRQ 550
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
501 SAMIAAIRDAKSANSAKSARDVAAGAGVSILLVDHEDSFVHTLANYFRQ 550
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

551 TGATVSTVRSPPAADVFDRFPDLVVLSPGPGSPTDFDCKATIKAAARARD 600
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
551 TGASVTTVRTPVAEEIFDRVKPDLVVLSPGPGPTPKDFDCKATIKKARARD 600
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

601 LPIFGVCLGLQALAEAYGGELRQLAVPMHGKPSRIRVLEPGLVFSGLGKE 650
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
601 LPIFGVCLGLQALAEAYGGDLRQLAIPMHGKPSRIRVLEPGLVFSGLGKE 650
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

651 VTVGRYHSIFADPATLPRDFIITAESDGTIMGIEHAKEPVAAVQFHPE 700
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
651 VTVGRYHSIFADPSNLPREFVITAESDGTIMGIEHSKEPVAAVQFHPE 700
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

701 IMTLGGDAGMRMIENVVHLTRKAKTKAA 729
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
701 IMTLGGDAGMRMIENVVAHLAKRAKTKAA 729
  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

```

Fig. 2

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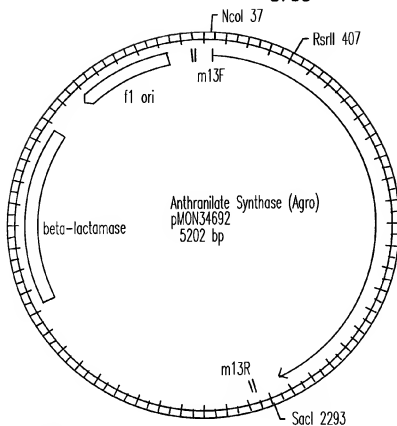


Fig. 3

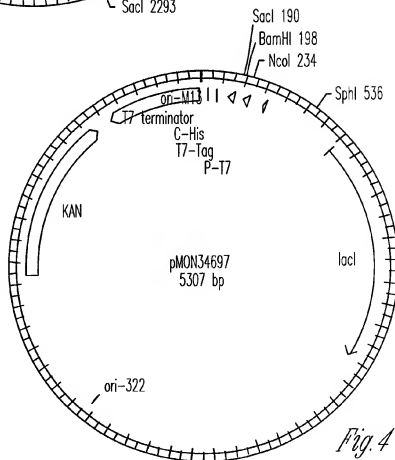
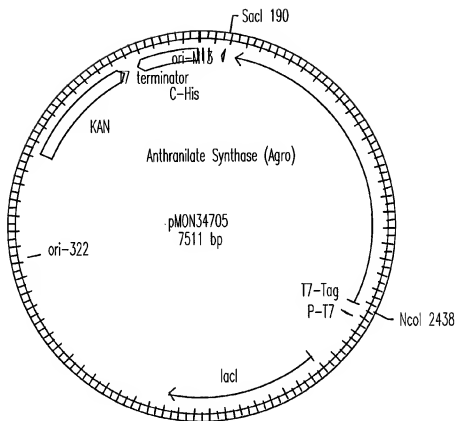


Fig. 4

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*Fig. 5*

Agrobacterium_TrpEG Sulfolobus_TrpE	MVTIIQDGAETKETKGGIQVSRKRRPTDYANAIDNYIEKLDHRGAVFSSNYEYPGRYT -----MEVHPISSEFASPEFVKIERDFKVAGLES-----
Agrobacterium_TrpEG Sulfolobus_TrpE	R-WDTAIVDPPLGISCFGRKMWIEAYNGRGEVLLDITEKLKATPDLTLGASSTRRLDLT -----IGGPQYKARYSVIAWSTNG-----YLKIHDDP-----VNIL
Agrobacterium_TrpEG Sulfolobus_TrpE	VNEPDRVTEERSKIPTVTALRAIVDLFYSSADSAGLFGAEGYDLAQFDAIKLSLA NG-----YLKDUKLADIPGLFKG-----GMIGYISYDAVRFWEKIRDLKP
Agrobacterium_TrpEG Sulfolobus_TrpE	RPEDORDMVLFLPDEILVVDHYSAKAWIDRYDFEKDGMTTDGKSSDITPDPFKTTDITPP AAEDWPYAEFTPDNIIIVDHNEGKVYN-----ADLSSVGGCGDIGEFKVSFYFDESIN
Agrobacterium_TrpEG Sulfolobus_TrpE	KGDHRPGEYSELVVKAKESFRRGDLFEVVPQGQFMERCESNPISAIRRLKAINPSPYSFT K--N--S-YERIVSELEYIRSGYIFQVVLRSFYRIFSGDPLRIYYNLRRLINPSPYMFY
Agrobacterium_TrpEG Sulfolobus_TrpE	INLGDAQYLVGASPEMFVRSGRRIETCPISGTIKRGDDPIADSEQILKLLNSKKDESEL LKF-DEKYLIGSSPELLFRVQDNIVETYPAGTRPRGADQEEDLKLEELMNSKDKAEH
Agrobacterium_TrpEG Sulfolobus_TrpE	TMCSDVDNDKSRVCEPGSVKVIIGRROIEWYSELHTVVDHIEGRLRDDMDADFDFLSHAW TMLVLDLARNDLGKVCVPGTVKVPMLMYVEKYSHVQHVIVSKVIGTLKKRYNALNVLSATFP

Fig. 6A

Agrobacterium TrpEG	AVTVTGAPKLWAMRFIEGHEKSPRAWYGGAIGMVGFGMDMNTGLTRTIRIKDGIAEVRA	6
Sulfolobus TrpE	AGTVSGRPKPMANNIETLEEYKRGPYAGAVGFISADGNAEFATAIRTAFLNKELLRIHA	53
Agrobacterium TrpEG	GATLLNDSNPQEEEAETELKASAMISAIRDAKGTNSAATKRDAAKVGTGVKILLVDHEDS	
Sulfolobus TrpE	GAGIVYDSNPESEYFETEHKLKALKTAIGVR-----	
Agrobacterium TrpEG	FVHTLANYFRQTGATVSTVRSVPVADVDFRQFDLVLSFGPGSPTDFDCKATIKAAAR	
Sulfolobus TrpE	-----	
Agrobacterium TrpEG	DLPIFGVCLGLQALAEAYGGELRLAVPMHGKPSRIRVLEPGLVFSGLGKEVTVGRYHSI	
Sulfolobus TrpE	-----	
Agrobacterium TrpEG	FADPATLPRDFIITAESDGTIMGIEHAKEPVAAVQFHPESIMTLIGQDAGMRMIENVVVH	
Sulfolobus TrpE	-----	
Agrobacterium TrpEG	LTRKAKTKAA	
Sulfolobus TrpE	-----	

Fig. 6B

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V48F-F: CCATCGCGCGCGTtTTTTTCGTCCAACtATG (SEQ ID NO:9)
 V48F-R: CATAGTTGGACGAAAAAaCGCGCCGCGATGG (SEQ ID NO:10)
 V48Y-F: CCATCGCGCGCGGtaTTTTTCGTCCAACtATGAATATCC (SEQ ID NO:11)
 V48Y-R: GGATATTCATAGTTGGACGAAAAAaCGCGCCGCGATGG (SEQ ID NO:12)
 V48W-F: CCATCGCGCGCGGtgTTTTTCGTCCAACtATGAATATCC (SEQ ID NO:13)
 V48W-R: GGATATTCATAGTTGGACGAAAAaCGCGCCGCGATGG (SEQ ID NO:14)
 S50K-F: CCATCGCGCGCGGTTTtaaGTCCAACtATGAATATCC (SEQ ID NO:15)
 S50K-R: GGATATTCATAGTTGGACttAAAAACCGCGCCGCGATGG (SEQ ID NO:16)
 S51C-F: GCGCGGTTTTTCGTgCAACTATGAATATCCGGG (SEQ ID NO:17)
 S51C-F: CCGGATATTCATAGTTGcACGAAAAAACCGCGC (SEQ ID NO:18)
 S51F-F: GCGGTTTTTCGTtCAACTATGAATATCCGGGC (SEQ ID NO:19)
 S51F-R: GCCCGATATTCATAGTTGaACGAAAAAACCGCGC (SEQ ID NO:20)
 S51I-F: CGGCGCGTTTTTTTCGatCAACTATGAATATCCGGGC (SEQ ID NO:21)
 S51I-R: GCCCGGATATTCATAGTTGatCGAAAAAACCGCGCC (SEQ ID NO:22)
 S51L-F: GCGCGCGTTTTTTTCGctCAACTATGAATATCCGGGC (SEQ ID NO:23)
 S51L-R: GCCCGGATATTCATAGTTGagCGAAAAAACCGCGCC (SEQ ID NO:24)
 S51M-F: CGGCGCGGTTTTTTTCGatgAACTATGAATATCCGGGCGC (SEQ ID NO:25)
 S51M-R: CGGCGCGGATATTCATAGTTcatCGAAAAAACCGCGCGC (SEQ ID NO:26)

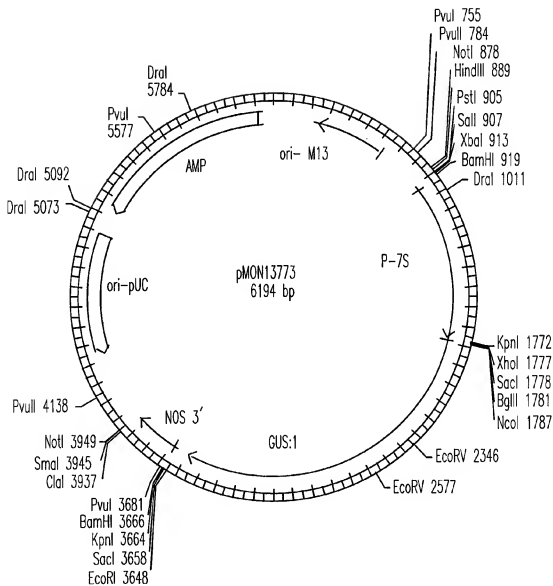
Fig. 7A

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S51T-F: CGCGGTTTTTCGaCCAACATATGAATATCCGGGC (SEQ ID NO:27)
S51T-R: GCCCGGATATTCATAGTTGGTCGAAAAAACCGCG (SEQ ID NO:28)
S51V-F: GGCGCGGTTTTTTTCGgtCAACTATGAATATCCGGGC (SEQ ID NO:29)
S51V-R: GCCCGGATATTCATAGTTGacCGAAAAAACCGGCC (SEQ ID NO:30)
S51Y-F GCGCGGTTTTTTTCGTaCAACTATGAATATCCGGGC (SEQ ID NO:31)
S51Y-R GCCCGGATATTCATAGTTGtACGAAAAAACCGCGC (SEQ ID NO:32)
N52F-F: CGGCGCGGTTTTTTTCGTCCcttCTATGAATATCCGGG (SEQ ID NO:33)
N52F-R: CCCGGATATTCATAGaaGGACGAAAAAACCGGCCG (SEQ ID NO:34)
P293A-F: CTGAAGGCGATCAACgCGTCGCCCTATTC (SEQ ID NO:35)
P293A-R: GAATAGGCGACGcGTTGATCGCCCTTCAG (SEQ ID NO:36)
P293G-F: CCTGAAGGCGATCAACggGTCGCCCTATTC (SEQ ID NO:37)
P293G-R: GGAATAGGCGACccGTTGATCGCCCTTCAGG (SEQ ID NO:38)
F298A-F: CGTCGCCCTATTCCgcCTTCATCAATCTCGGC (SEQ ID NO:39)
F298A-R: CGCCGAGATTGATGAAGcCGGAATAGGCGGACG (SEQ ID NO:40)
F298W-F: CGTCGCCCTATTCCtGgTTCATCAATCTCGGC (SEQ ID NO:41)
F298W-R: CGCCGAGATTGATGAAccAGGAATAGGCGGACG (SEQ ID NO:42)

Fig. 7B

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*Fig. 8*

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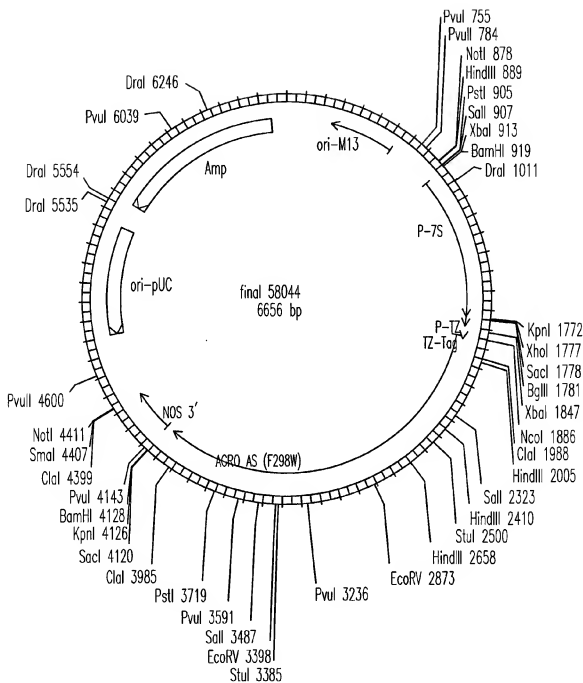


Fig. 9

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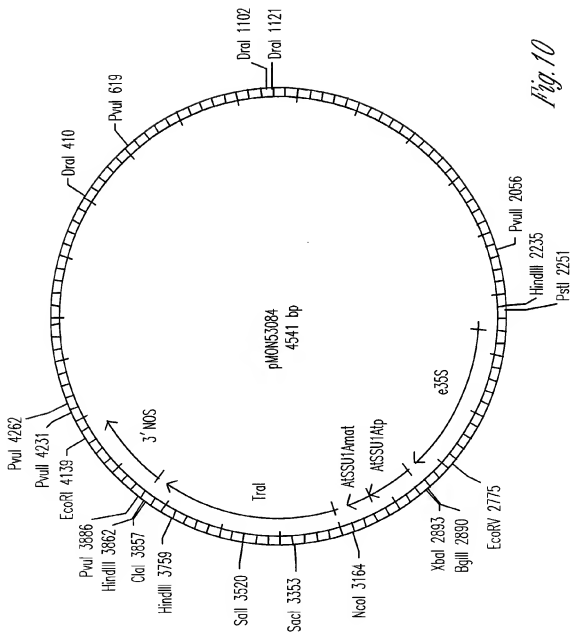


Fig. 10

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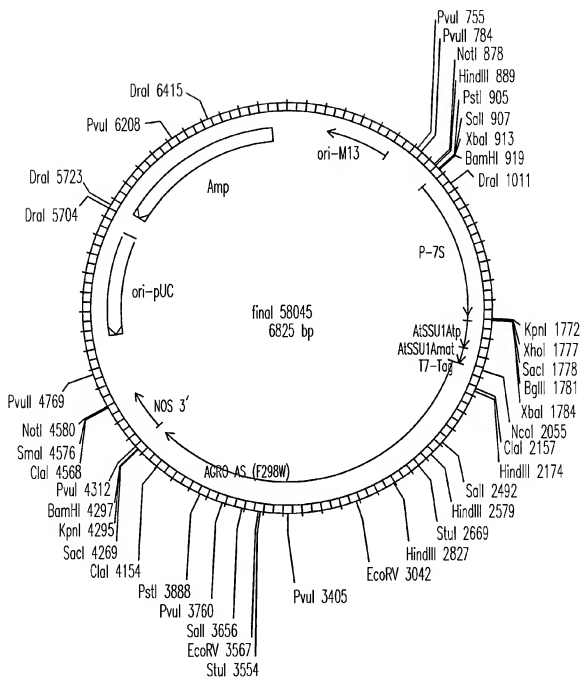


Fig. 11

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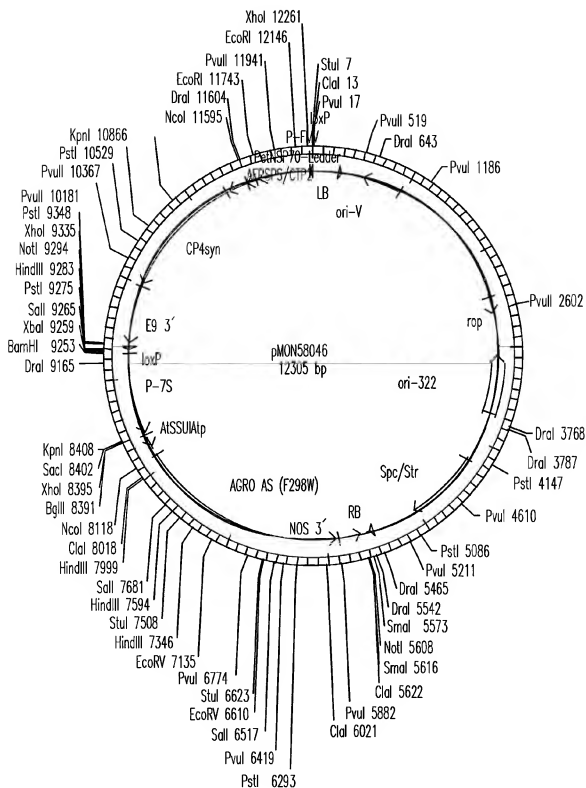


Fig. 12

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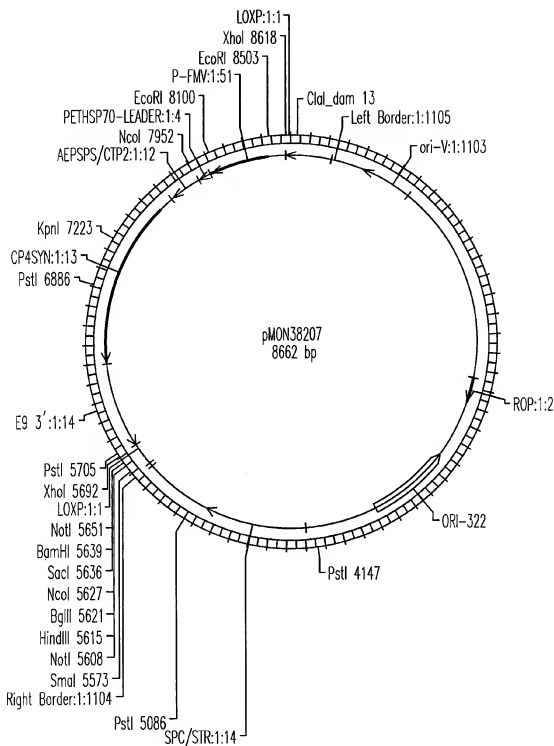


Fig. 13

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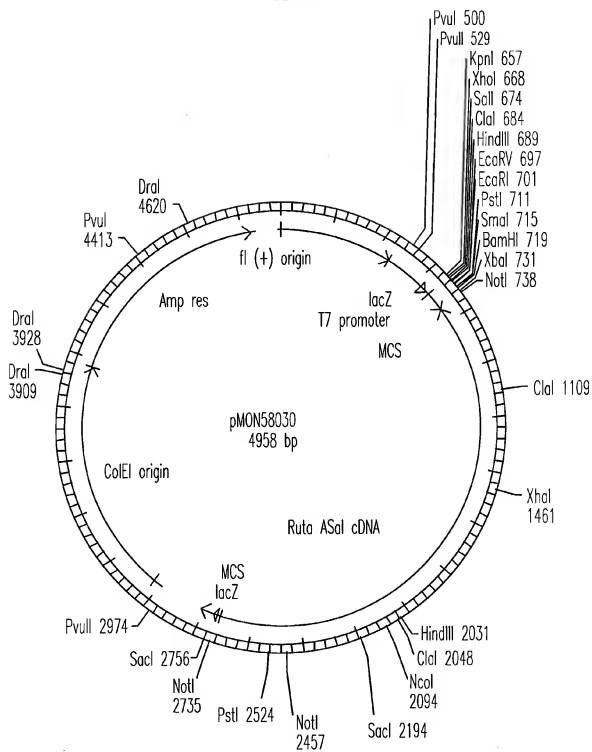
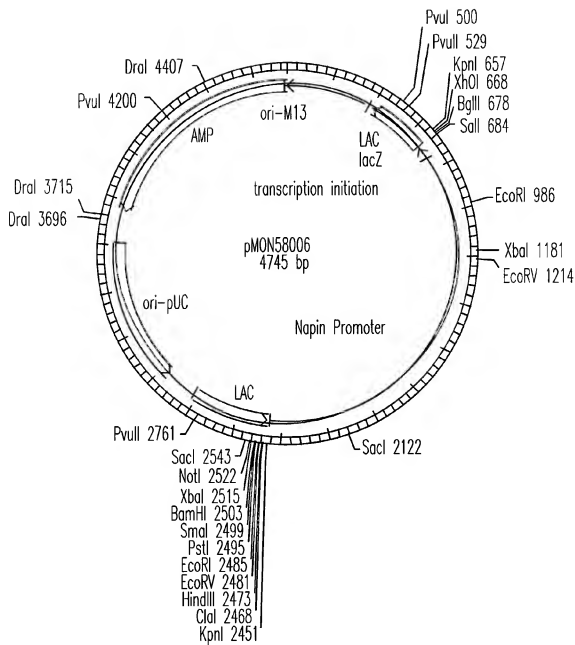


Fig. 14

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*Fig. 15*

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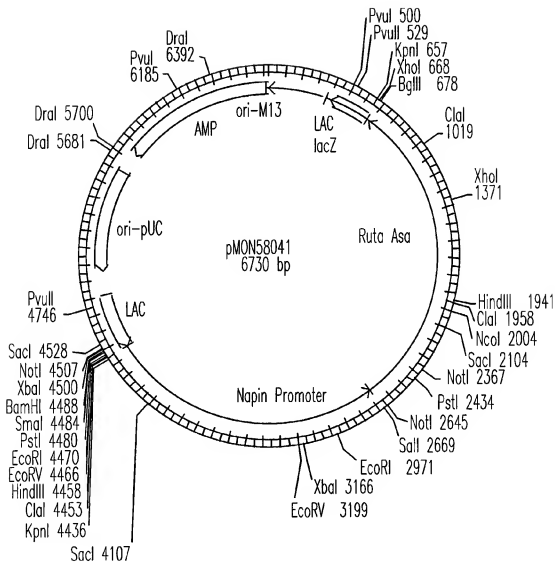


Fig. 16

18/53

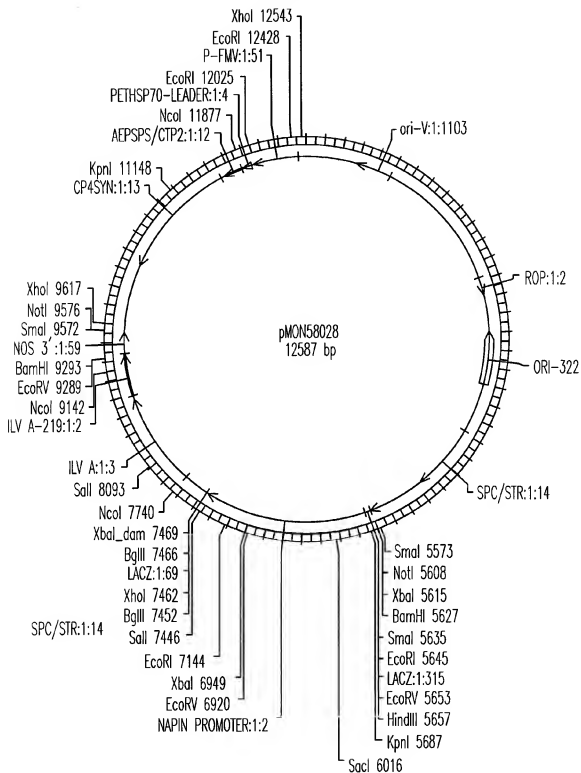


Fig. 17

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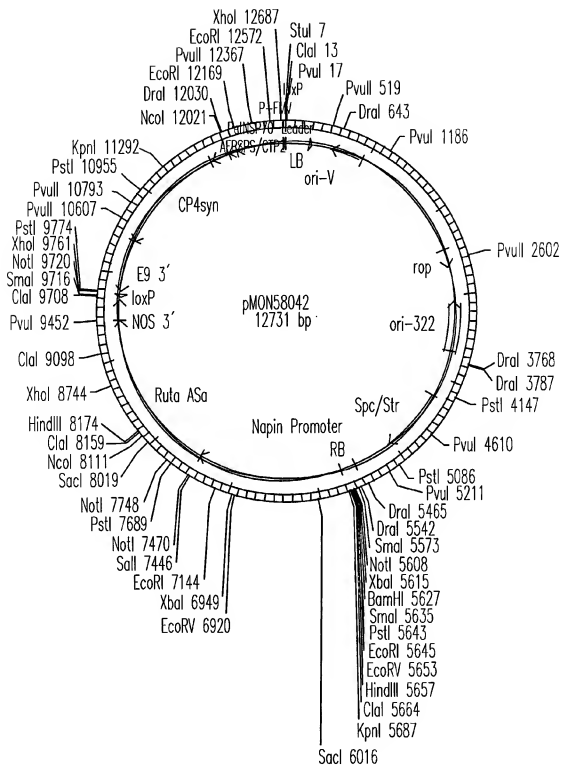


Fig. 18

20/53

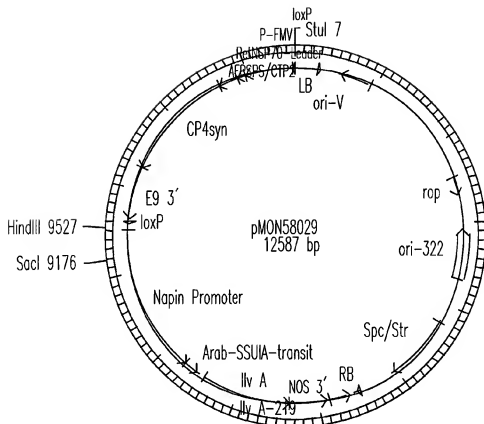


Fig. 19

21/53

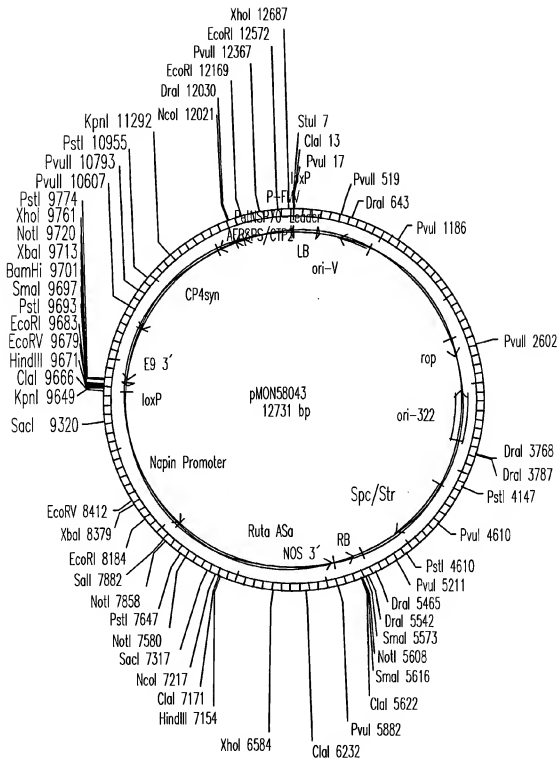


Fig. 20

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```

TRPEG_AGRU_MONSANTO_
TRPEG_RHIME_A30904_
TRPE_SULSO_Q06128_
TRPE_ARATH_S27752_
27
MAAVILEDGAESYTKGGIVTRRRR-----27
MEVHPISEAFSPFEVKCIER-----21
MSAVSISAVKSDFFVEAIAVTHRTPHPPHFPFLSLKSPATS LN 50
* . . . . *
27
TDYANAIDNYIEKLSHRGAVFSSNYEYPGRYTRWDTAIVDPPLGISCFG 77
ASYSDAIAGYVDRDLERRGAVFSSNYEYPGRYTRWDTAIVDPPLAISFG 77
-----DFKVAG-----27
LVAGSKLLHFRRRLPSIKCSYTPSLDLSEEQFTKFKKASEKGNLVLFRCL 100
. . *
-----RKMWIEAYNGRGEVLLDFITEKLLKATPDLTLGASSTR 115
-----RSLWIEAYNERGEVLLALIAEDLKSVADITLGSIAARR 115
-----LLESIG-GPO--YKARYSVIAWST 48
VFSDDLTPILAYRCLVKEDDRDAPSFLESVEPGSQSSNIGRYSVYGAQP 150
* . . . .
LDLTVNEPDRVFTE-----EERSKIPTVTALRAIYVDLFYSSA 153
LDLTINEPDRVFTE-----EERSKMPTVFTVLRVATNLFHSEE 153
NGYLKTH-----DDPVNIIINGYLKDLK--LADIPG 76
TTEIVAKGNVVTVMDHGASLRTEBEVDDPMVPQKINEEWNPGIDELPE 200
. . . .
DSAIGLFGAGFYDLAFQFDAIKLSIARPELQ-----RDMVLFPLFDEILVVD 199
DSNLGLYGAFGYDLAFQFDAIELKSRPDDQ-----RDMVLFPLFDEILVVD 199
LFGKGMIGYISYDAVRFWEKIR-DLKPAED-----WPYAEFFTPDNIILYD 122
AFCGGWGIFYSDYTRYVYRVEKKLPPFSNAPEDDRSLPDVNLGLYDDVIVFD 250
* * * . . . . . *

```

Fig. 21a

```

TRREG_AGRTO_MONSANTO_-----222
TRREG_RHIME_A30904_-----148
TRPE_SULSO_Q06128_-----228
TRPE_ARATH_S27752_-----300
HYSAKAWIDRYDEKDGMTTDG-----KSSDITP-
HYAKAWIDRYDFARENLSTEG-----KAADTAP-
HNECKYVN-----ADLSSVG-----CGCDTG-
HVEKAYVIHWVRIDKDRSVEENFREGMNRLESLSRIQDKPKMPTGF
* * * * *
--DPFKTTDTIPKGDHRPGEYSSELVVKAKESFRRGDLFEVVPQKFMER 276
--EFRSVDSPHIGDDHRPGEYAEVLVVKAKESFRRGDLFEVVPQKFRYI 276
--EFKVSDESINLK-----NSVERIVSELSLEYRISGYFVQVLSQFYRI 188
IKLRTQLFGPKLEKSTWTSEAYKEAVVEAKEHILAGDIFQVLSQPFERR 350
* * * * *
CESNPSAISRRLKAINPSPYFIFNLGDOEYLVGASPEMFVRVSGRRIET 326
CESPSEISNRLKAINPSPYFIFNLGDOEYLVGASPEMFVRVSGRRIET 326
FSGDPLRIYNLRLAINPSPYFMFYKFD-EKYLLIGSPELLEFPVQDNIVET 237
TFADPEIYRALRIYNPSPYMAVLOVR-GCILVASSPEILLRSKNRKITN 399
* * * * *
CPISTIKRGDDPIADSEQILKLLNSKKDESELITMCSDDVRDNDKSRVCEP 376
CPISTIKRGDDPIADSEQILKLLNSKKDESELITMCSDDVRDNDKSRVCEP 376
YPIATGRPGADQEDLKLLELNSKDKAHLMLVDLARNLDGLKVCVP 287
RPLAGTVRAGKTPKEDLMLEKLLSDBKQCAEHIMLVLDLGRNDVGRVSKP 449
* * * * *
GSVKVIGRRQIEMYSRLIHTVDHIEGRURDDMDAFDGLSHAWAVTGTGA 426
GSVKVIGRRQIEMYSRLIHTVDHIEGRURDDMDAFDGLSHAWAVTGTGA 426
GTVKVPELNVYEKYSHVQIHVSKVIGTLKKKNYNALNVLSTAFPAGTVSGR 337
GSVBVKKLKDIEWPSSHVMHISSTTVVGEILLDHLTISWDALRAVLVPGVTSGA 499
* * * * *
TRREG_AGRTO_MONSANTO_-----
TRREG_RHIME_A30904_-----
TRPE_SULSO_Q06128_-----
TRPE_ARATH_S27752_-----
TRREG_AGRTO_MONSANTO_-----
TRREG_RHIME_A30904_-----
TRPE_SULSO_Q06128_-----
TRPE_ARATH_S27752_-----
TRREG_AGRTO_MONSANTO_-----
TRREG_RHIME_A30904_-----
TRPE_SULSO_Q06128_-----
TRPE_ARATH_S27752_-----
TRREG_AGRTO_MONSANTO_-----
TRREG_RHIME_A30904_-----
TRPE_SULSO_Q06128_-----
TRPE_ARATH_S27752_-----

```

Fig. 21B

[illegible]

Fig. 21C

[illegible]

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Fig. 21D

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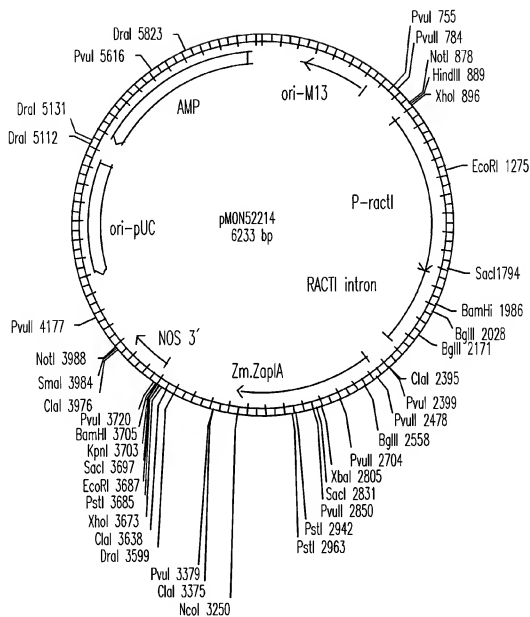


Fig.22

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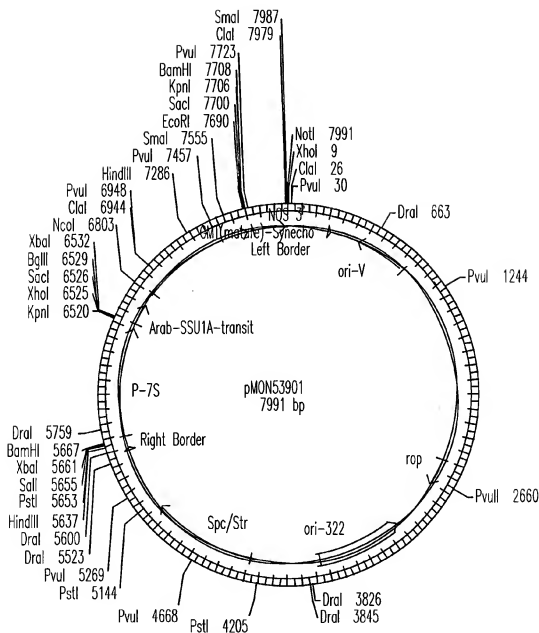


Fig. 23

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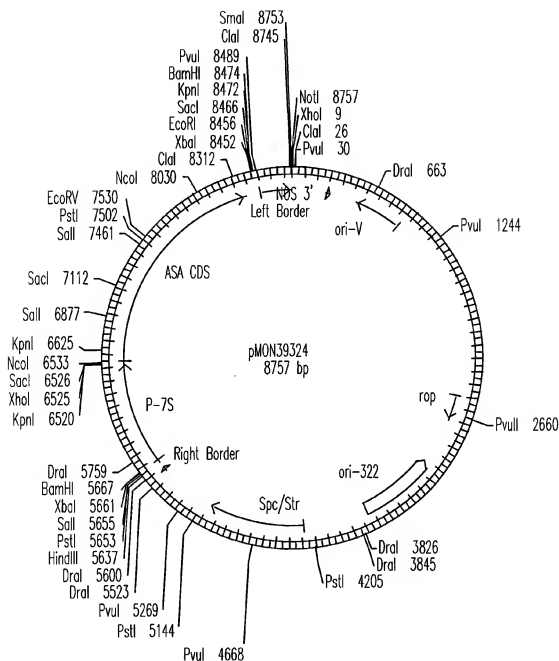


Fig. 24

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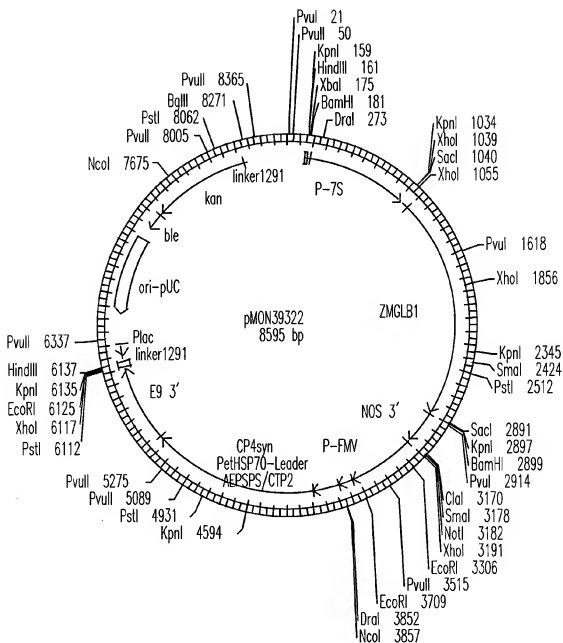


Fig. 25

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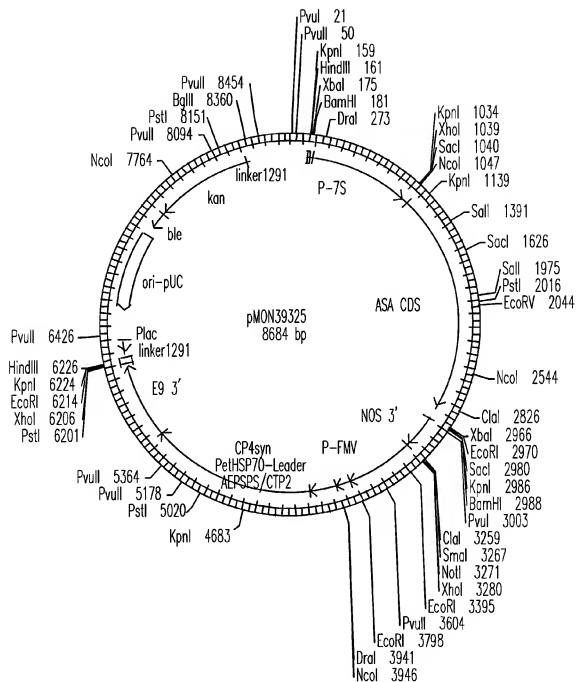


Fig.26

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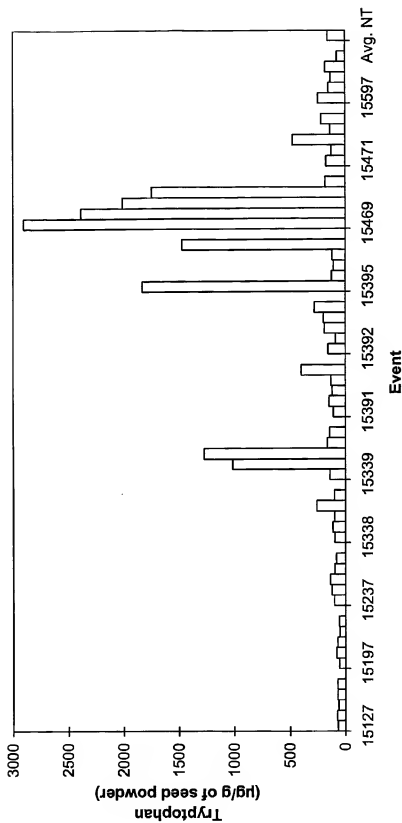


Fig. 27

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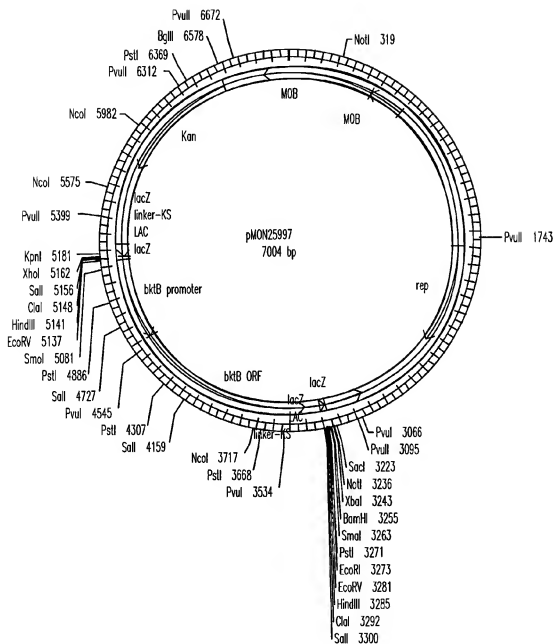


Fig. 28

33/53

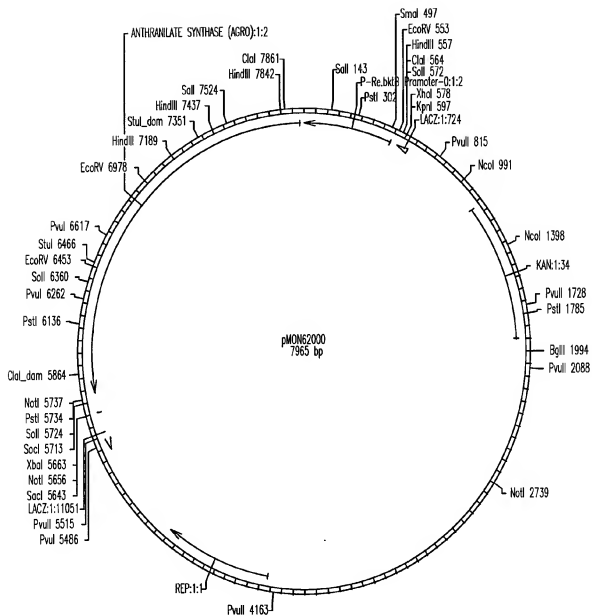


Fig.29

atgcaaacacaaaaaccgactctcgaaactggaaattcctgggtggaacggtatcgccaccgt
gcaagcgggtgctggtgtagtccttgattctgttccgcagtcggaagccgacgaaaacccgta
acaaagcccgcgtgtactgcgcgtattgccaccgcgcattgcacaggagactttctga
tggctggacattctgctgctcgataatatcgactcttttacgtacaaacctggcagatcagtt
gcgca

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Fig. 30

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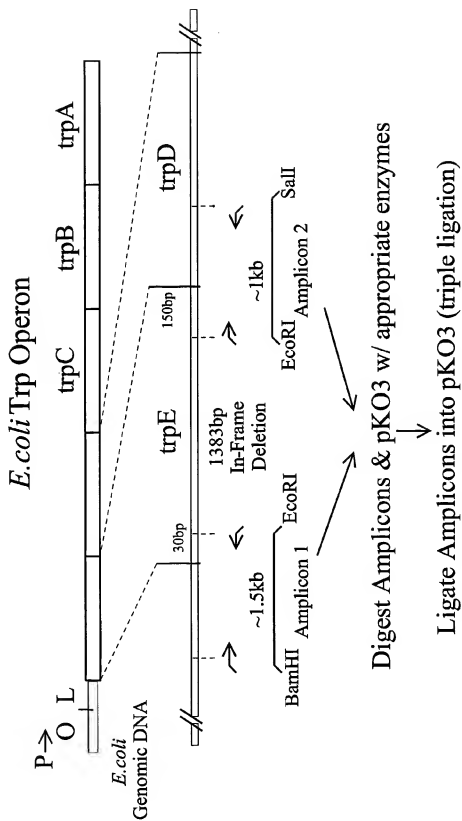


Fig. 31

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1 - ATGTTAACGATCATTCAGGATGACGGAGCGGAGACCTACGAGACGAAAGCGGCATCCAG - 60
 - M V T I I Q D D G G A E T Y E T K G I Q
 61 - GTCAGCGGAAGCGCGGCCACCGATTATGCCAACGCCCATGATAATTACATCGAAAG - 120
 - V S R K R P T D Y A N A I D N Y I E K
 121 - CTTGATCCCATCGCGCGGGTTTTTCGTCCAACTATGAAATATCCGGGCGGTACACC - 180
 - L D S H R G A V F S S N Y E Y P G R Y T
 181 - CGCTGGATACGCCCATCGTCGATCCGCGCTCGGCATTTCTGTGTTTGGCGCAAGATG - 240
 - R W D T A I V D P P L G I S C F G R K M
 241 - TGGATCGAAGCCTATAATGGCGCGGCGGAAGTGTCTCGATTTCATTACGSAAGAGCTG - 300
 - W I E A Y N G R G E V L L D F I T E K L
 301 - AAGCGACACCGGATCTCACCTCGGGCTTCCTCGACCCGCGGCTCGATCTTACCGTC - 360
 - K A T P D L T L G A S S T R R L D L T V
 361 - AACGAACCGGACCGTGTCTTCACCGAAGAAACGCTCGAAATCCCGACGGTCTTCACC - 420
 - N E P D R V F T E E R S K I P T V F T
 421 - GCTCTCAGAGCCATCGTCGACCTCTTCTATTGAGCGGGATTCGGCCATCGGCCTGTT - 480
 - A L R A I V D L F Y S S A D S A I G L F
 481 - GGTGCCTTCGTTACGATCTCGCCTTCCAGTTCGACGCGATCAAGCTTTCGCTGGCGCGT - 540
 - G A F G Y D L A F Q F D A I K L S L A R
 541 - CCGAAGACCGCGTGACATGCTGTGTTTCTGCCGATGAATCTCTCGTTGATCAC - 600
 - P E D Q R D M V L F L P D E I L V D H
 601 - TATTCGCCAAGCCCTGGATCGACCGTTACGATTTCGAGAAGGACGGCATGACGACGGAC - 660
 - Y S A K A W I D R Y D F E K D G M T T D
 661 - GGCAATCCTCCGACATTACCCCGGATCCCTTCAAGACCCGATACCATCCCGCCCAAG - 720
 - G K S S D I T P D P F K T T D T I P P K
 721 - GCGATCACCGTCCCGGGAATATTCCGAGCTTGTGTGAAGCCCAAGGAAGCTTCCGC - 780
 - G D H R P G E Y S E L V V K A K E S F R
 781 - CGCGGCGACCTGTTTCGAGGTTCGTTCCCGGCGCAAAATTCATGAGCGGTTCGSAAGCAAT - 840
 - R G D L F E V V P G Q K F M E R C E S N

Fig 32A

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841 - CGCTCGGCGGATTTCCGCGCGCTGAAGCGGATCAACCGTCGCGCCTATTCTCTTCTCATC - 900
 - P S A I S R R L K A I N P S P Y S F F I
 901 - AATCTCGGGATCAGGAATATCTGGTCGGCGCTCGCGGAAATGTTCTGGCGGCTCTCC - 960
 - N L G D Q E Y L V G A S P E M F V R V S
 961 - GGCGTCGATCGAGACCTGCCGATATCAGGCACCATCAAGCGGCGGACGATCCGATT - 1020
 - G R R I E T C P I S G T I K R G D D P I
 1021 - GCCGACAGCGAGCATTTTGAACCTGCTCAACTCGAAAAGGACGAATCCGAACCTGACC - 1080
 - A D S E Q I L K L L N S K K D E S E L T
 1081 - ATGTGCTCGAGCTGGACCGCAAGACAGACCGCGCTCGAGACCGGTTTCGGTGAAG - 1140
 - M C S D V D R N D K S R V C E P G S V K
 1141 - GTCATTGGCGCGCCGAGATCGAGATGTATTCAACGCCCTCATCCACCCGTCGATCACATC - 1200
 - V I G R R Q I E M Y S R L I H T V D H I
 1201 - GAAGGCCGCTGCGCGACGATATGGACGCCCTTTGACGGTTTCTCAGCCACGCTGGGC - 1260
 - E G R L R D D M D A F D G F L S H A W A
 1261 - GTCACCGTCACCGGTGACCAAGCTGTGGCCATGCGCTTCTATCGAAGTCTATGAAAG - 1320
 - V T V T G A P K L W A M R F I E G H E K
 1321 - AGCCGCGCGCCTGGTATGGCGGTGCGATCGGCATGGTCGGCTTCAACGCGGACATGAAT - 1380
 - S P R A W Y G G A I G M V G F N G D M N
 1381 - ACCGGCTACGCTGGCACCATCCGGATCAAGGACGGTATTGCCGAAGTGGCGCGCGC - 1440
 - T G L T L R T I R I K D G I A E V R A G
 1441 - GGGACCTGCTCAATGATTCGAACCGCGAGGAAGAGCCGGAACCGAAGTGAAGGC - 1500
 - A T L L N D S N P Q E E A E T E L K A
 1501 - TCGCCATGATATCAGGCATCTGTGACGCAAGGACCAACTCTCCGCCACCAAGCGT - 1560
 - S A M I S A I R D A K G T N S A A T K R
 1561 - GATCCGCAAGTTCGGACCGGCTCAAGATCTGTCTGTCGACACGACGACAGCTTC - 1620
 - D A A K V G T G V K I L L V D H E D S F
 1621 - GTGCACACGCTGGCGAATATTTCGCCAGAGGGCGGCGACGGTCTCGACCGTCAGATCA - 1680
 - V H T L A N Y F R Q T G A T V S T V R S

Fig. 32B

1681 - CCGGTCGACGCCGACGTGTTTCGATCGTTCCAGCCGGAACCTCGTGTTCCTGTCCGCCCGGA - 1740
 - P V A A D V F D R F Q P D L V V L S P G
 1741 - CCCGGACGCCGACGGATTTCGACTGCAAGCAACGATCAAGCGCCCGCGCCCGCGGAT - 1800
 - P G S P T D F D C K A T I K A A R A R D
 1801 - CTGCCGATCTCGGCGTTTGCCCTCGGTCTGCAGGCATGGCAGAAGCCATGGCGGCGAG - 1860
 - L P I F G V C L G L Q L A E A Y G G E
 1861 - CTGCGCCAGCTTGCTGTGCCCCATGCACGGCAAGCCTTCGCGCATCCGCGTGTGGAACCC - 1920
 - L R Q L A V P M H G K P S R I R V L E P
 1921 - GGCTCGTCTTCTCCGGTCTCGCAAGGAAGTCAAGTCGGTTCGTTACCATTCGATCTTC - 1980
 - G L V F S G L G K E V T V G R Y H S I F
 1981 - GCGATCCCGCCACCCCTGCGCGTGATTTCATCATCACCGCAGAAGCGAGACGGCAG - 2040
 - A D P A T L P R D F I I T A E S E D G T
 2041 - ATCATGGGATCGAACACGCCAAGCAACCGGTGGCGCGTTTCAGTTCACCCGGAATCG - 2100
 - I M G I E H A K E P V A A V Q F H P E S
 2101 - ATCATGACGTCGGACAGGACGGGCGATGCGGATGATCGAGAATGTCGTGGTGCACTG - 2160
 - I M T L G Q D A G M R M I E N V V V H L
 2161 - ACCGCAAGGCGAAGACCAAGCCGCGTGA - 2190
 - T R K A K T K A A *

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Fig. 32C

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1 ATGGAATCCC TAGCCGCCAC CTCCGTGTTT GGGCCCTCCC GCGTCGCCGT
51 CCCGCGGCG CGGGCCCTGG TTAGGGCGGG GACGGTGGA CCAACAGGC
101 GGACGAGCAG CCGGAGCGGA ACCAGCGGG TGAATGCTC TGCTGCCGTG
151 ACGCGCAGG CGAGCCCAGT GATTAGCAG AGCGTGCGG CGGCGAAGGC
201 GCGGAGGAG GACAAGAGG GGTTCCTCGA GCGGGCGGG CGGGGGAGCG
251 GGAAGGGGA CCTGGTGCC ATGTGGAGT GCATCGTGT GGACCATCTC
301 ACCCCCGTGC TCGCCTACCG CTGCCCTCGT CCCGAGGACA ACGTCGACGC
351 CCCCAGCTTC CTCTTCGAGT CCGTCGAGCA GGGGCCCCAG GGCACCAACA
401 ACGTCGGCG CTATAGCAT GTGGGAGCCC ACCAGTGAT GGAGATTGTG
451 GCCAAAGACC ACAAGGTTAC GATCATGGAC CACGAGAAGA GCCAAGTGAC
501 AGAGCAGGTA GTGACGACC CGATGCAGT CCCGAGGACC ATGATGGAGG
551 GATGCACCC ACAGCAGATC GACGAGCTCC CTGAATCCTT CTCGGGTGGA
601 TGGGTTGGGT TCTTTTCCTA TGATACGGTT AGGTATGTTG AGAAGAAGAA

Fig. 33A

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651 GCTACCGTTC TCCAGTGTCT CTCAGGACGA TAGGAACCTT CCTGATGTGC
701 ACTTGGGACT CTATGATGAT GTTCTAGTCT TCGATAATGT TGAGAAGAAA
751 GTATATGTTA TCCATTGGGT CAATGTGGAC CGGCATGCAT CTGTTGAGGA
801 AGCATACCAA GATGGCAGGT CCCGACTAAA CATGTTGCTA TCTAAAGTGC
851 ACAATTCCAA TGTCCCCACA CTCTCTCCTG GATTGTGAA GCTGCACACA
901 CGCAAGTTTG GTACACCTTT GAACAAGTCG ACCATGACAA GTGATGAGTA
951 TAAGAATGCT GTTCTGCAGG CTAAGGAACA TATTATGGCT GGGGATATCT
1001 TCCAGATTGT TTTAAGCCAG AGGTTGAGA GACGAACATA TGCCAACCCA
1051 TTTGAGGTTT ATCGAGCATT ACGGATTGTG AATCCTAGCC CATACATGGC
1101 GTATGTACAG GCAAGAGGCT GTGTATTGGT TCGGTCTAGT CCTGAAATTC
1151 TTACACGAGT CAGTAAGGGG AAGATTATTA ATCGACCACT TGCTGGAAC
1201 GTTCGAAGGG GCAAGACAGA GAAGGAAGAT CAAATGCAAG AGCAGCAACT
1251 GTTAAAGTGAT GAAAAACAGT GTGCCGAGCA CATAATGCTT GTGGACTTGG

Fig. 33B

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1301 GAAGGAATGA TGTGGCAAG GTATCCAAAC CAGGATCAGT GAAGGTGGAG
1351 AAGTTGATGA ACATTGAGAG ATACTCCCAT GTTATGCACA TCAGTCAAC
1401 GGTAGTGA CAGTTGGATG ATCATCTCCA GAGTTGGAT GCCTTGAGAG
1451 CTGCTTGCC CGTTGGAACA GTCAGTGGTG CACCAAAGGT GAAGGCCATG
1501 GAGTTGATTG ATAAAGTTGGA AGTTACGAGG CGAGGACCAT ATAGTGGTGG
1551 TCTAGGAGGA ATATCGTTTG ATGGTGACAT GCAAATTGCA CTTTCTCTCC
1601 GCACCATCGT ATTCTCAACA GCGCCGAGCC ACAACACGAT GTACTCATAC
1651 AAAGACGCAG ATAGGCGTCG GGAGTGGTC GTCATCTTC AGGCTGGTGC
1701 AGGCATTGTT GCCGACAGTA GCCCAGATGA CGAACAACGT GAATGCCAGA
1751 ATAAAGGTGC TGCACTAGCT CGGGCCATCG ATCTTGCAGA GTCAGCTTTT
1801 GTAGACAAAG AATAG

Fig. 33C

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1 MESLAATSVF APSRVAVPAA RALVRAGTVV PTRRTSSRSR TSGVKCSAAV
51 TPQASPVISR SAAAAKAAEE DKRRFFEEAA RGSGKGNLVP MWECIVSDHL
101 TPVIAYRCIV PEDNVDAPSF LFESVEQGPQ GTTNVGRYSM VGAHPVMEIV
151 AKDHKVTIMD HEKSQVTEQV VDDPMQIPRT MMEGWHPQOI DELPESFSGG
201 WVGFFSYDTV RYVEKKKLPF SSAPQDDRNL PDVHLGLYDD VLVFDNVEKK
251 VYVIHWNVND RHASVEEAYQ DGRSLNMLL SKVHNSNVPT LSPGFVKLHT
301 RKFGTPLINKS TMTSDEYKNA VLQAKEHIMA GDIFQIVLSQ RFERRTYANP
351 FEVYRALRIV NPSPYMAYVQ ARGCVLVASS PEILTRVSKG KLINRPLAGT
401 VRRGKTEKED QMQEQQLSD EKQCAEHIML VDLGRNDVGK VSKPGSVKVE
451 KLMNIERYSH VMHISSTVSG QLDDHLQSWD ALRAALPVGT VSGAPKVKAM
501 ELIDKIEVTR RGPYSGGLGG ISFDGDMQIA LSLRTIVFST APSHNTMYSY
551 KDADRRREWV AHLQAGAGIV ADSSPDDEQR ECENKAAALA RAIDLAESAF
601 VDKE*

Fig. 33D

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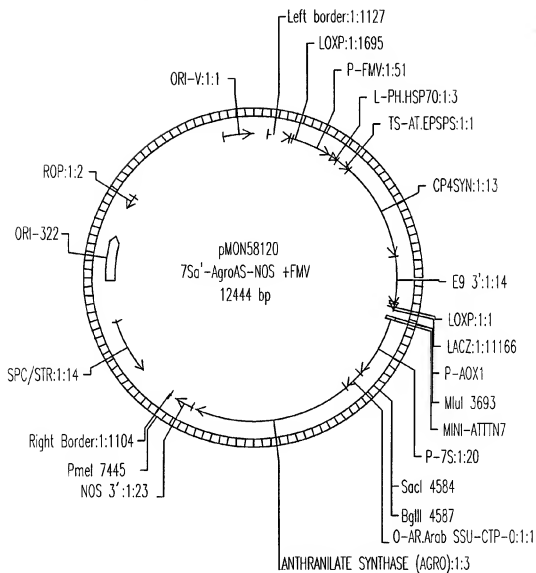


Fig. 34

AgRtu	15889565	-----MVTIIQDGAETVETKGGIQVSRKRPPTYDANAI DNYIEKLDHSRGVAFSSNVEY	55
RhIme	136328	-----MAAIVLDGAESYTKGII VTRRREASYPADAIAGYVDRLDERRGAVFSSNVEY	55
MesLo	13472468	---METAMTKVLENGAESYFTAGGII ITRERHDPYAGADAIYDGLNSRRGAVFSSNVEY	59
AzoBr	171765	MYPADLLASPDIIJELPRQTRGGVTVRRATALDPTALDPVIDALDRRGLLLSSGVEA	60
BruMe	17986732	-----MNAKTADSEIIFQETAGGII VERVRHLTKAGAESYIIVLWNRGAVFSSNVEY	55
Nostoc	17227910	-----MADSHSYRTNGNVRSR IITQVKMETALEEILFVLNSQRGGLLTSVVEY	50
Nostoc	17230725	-----MRVSRSTTEYKMDTALDEILFHLNQRGGLLTSVVEY	37
RhoPa_TpEG		---MNRVFSLPATSDYKTAAGLAVTSAQPPAGGOALDELIDLLDHRRGVMLSSGGTIV	56
AgRtu	15889565	PGRYTRWDTAIVDPPLAISGFCGRKMWIEAYNGRGEVLLDFITEKLCATPDLTIGCSLAARR	115
RhIme	136328	PGRYTRWDTAIVDPPLAISGFCGRKMWIEAYNGRGEVLLALIAEKLKSVADITIGCSLAARR	115
MesLo	13472468	PGRYTRWDTAIIDPPLAISARGRAMRIEALNRGEALLPVIGTKLGGADITIAETTTTL	119
AzoBr	171765	PGRYRRHALGFTDPAAVALTARGKTRLRIDALNGRQVILPAVAEARGLGLEALGLEEAPSR	120
BruMe	17986732	PGRYTRWDTAIVDPVPVITSRAKTRIEALNARGVILRLPILDTIKVASEVKIDQSGHNK	115
Nostoc	17227910	PGRYKRWAI GFVNPPVELSTSQTFTLILNARGCVLLPVIFECLSKSKLOKLTGHEHKK	110
Nostoc	17230725	PGRYKRWAI GFVNPPQLTTRENAFITSSLNPRQVLLTFLFOHLSAOSOLOQILSNHDY	97
RhoPa_TpEG		PGRYESFDLGADFPPLAULTTRAEKFTIEALNPRGVLIATFLSKLEEPCVWVEQACATKI	116
AgRtu	15889565	LDLTINEPDRVTFTEERSKIPVTFTALRAIVDLFYSSADSNAIGLGFAGFYDLAQFDAIK	175
RhIme	136328	LDLTINEPDRVTFTEERSKMPVFTVLRVATNLFHSEEDSNLIGLYGAFGYDLAQFDAIE	175
MesLo	13472468	IRLDVAKRGVTFTEERSKRVPSFTVLRATITLFTKDEDANLIGLYGAFGYDLQSFPDVP	179
AzoBr	171765	VTASSASAPL-PEEERSKQPSVSVLRVLDLFAAPDDPLIGLYGAFAYDLAQFQEPFR	175
BruMe	17986732	LDLTIVEBGTTFTEERSKMPSTVTLRAIVLGFVSEEDANLIGLYGAFGYDLAQFQDPTQ	175
Nostoc	17227910	ITGLVKSPTFEFFAEERSKQPSFTVIREILHFPSSQEDHEHLGYGAFGYDLVQFQETQ	170
Nostoc	17230725	ITGFIRPKQLFTFEERQSKQPSFTVIREILOIFASDEDEHLGYGAFGYDLVQFQEPFP	157
RhoPa_TpEG		RGHIVRGAPV-DEEQRTTRASALSVLRVAVIAPASDPMLGILGAYADLVQFQEDLUK	175

Fig. 35A

[illegible]

Fig. 35B

AgriTu_15889565	GKVLIVDHEDSEVHTTLANIYFROTGA TVSTVRSPVAADVDRQFPDLVVLSPPGSGPDF	587
RhiMe_136328	GVSILLVDHEDSEVHTTLANIYFROTGAN SVTTTRPVAERI FDRVKLDPDVLLSPGGTPKDF	587
MesLo_13472468	GNNILVDHEDSEVHTTLANIYFROTGANS TVRPDVBFEVRKLDPDVLLSPGGTPKDF	591
AzoBr_1717765	GRVLLVDHDDSEVHTTLADYLROTGAS VTTLRSHARAALAERRPDVVLSPPGGRPADF	590
BruMe_17986732	GVSILLVDHEDSEVHTTLANIYFROTKAVTS TVRSPVAERI FDRVNPDLVVLSPPGSPQDF	587
Nostoc_17227910	GKHILLVDHEDSEVHTTLANIYIRSTGAT VTTLRHGFSSELDETERKPDVVLSPPGPRSEF	589
Nostoc_17230725	NKRILLIDYEDSEVHTTLANIYRTGATV TTTLRHGFAES FDEARPDVVLSPPGGRPSDF	571
RhoPa_TpeG	GRKVLLVDHDDSEVHMMLADFYRQGVQTV RRVXVHGLKMLAENS DLLIVLSPGGRPSDF	584
	. :***.:***** ***:***.:***.:***.:***.:***.:***.:***.:***.:***.	
AgriTu_15889565	DCKATIKAARADLP IFGVCLGLQALAEAYGGDLRLQ LPAVMHGKPSRIRVLEP-GLVFSG	646
RhiMe_136328	DCKATIKAARADLP IFGVCLGLQALAEAYGGDLRLQ LAIPMHGKPSRIRVLEP-GIVFSG	646
MesLo_13472468	DCOAATIRARADLP IFGVCLGHQALAEAYGGEQLHL IPMHGKPSRIRVSKP-GIIFSG	650
AzoBr_1717765	DVAGTIDAALLGIPIFGVCLQGWMFRFGLADLV LPPEPHGKATVEYVLVG--ALFAG	648
BruMe_17986732	DCKATIKAARKROLPIFGVCLGLQALAEAYGGALRLQ LVNVPVHGKPSRIRVSKP-ERIFSG	646
Nostoc_17227910	KVQETVAACRRQIPLFI GVCVLGHQIVAEAFGBELGVN PQHGKSSRIFTVAPDSVMFQD	649
Nostoc_17230725	RVPTVAALVGREIPIFGVCLGLQIGVBAFGGELGVLD YPOHGKPARISVTAPDSVLFN	631
RhoPa_TpeG	KIKDTIDAALLAKLP IFGVCLGVQAMGEYFGGTILGQLAQ PAHGPRPSRIQVRGG--ALMRG	642
	*:*****:***** ***:***.:***.:***.:***.:***.:***.:***.:***.:***.	
AgriTu_15889565	LKGEVTVGRYHSIFADPATLPROFLITAE SEDGTIMGIEHAKEPVAAVQFHPPSIMTLGQ	706
RhiMe_136328	LKGEVTVGRYHSIFADPSNLPREFVI TAESEDGTIMGIEHSKEPVAAVQFHPPSIMTLGQ	706
MesLo_13472468	LKPEVTVGRYHSIFADPVRLPDOLF VTAAETEDGT IMATHEKRKP IAAVQFHPPSIMTLGH	710
AzoBr_1717765	LPERLTVGRYHSILVARDELPA DLTVAETAADGLVMAVEHRRRLPAAVQFHPPSILSDG	708
BruMe_17986732	LPEEVTVGRYHSILVARDELPA DLTVAETAEDGT IMAFETHKEPVAVQFHPPSIMTLG	706
Nostoc_17227910	LPESFTVGRYHSILFALSOKLP KLVTAISDDEVIMATEHOTLP IAAVQFHPPSIMTLAG	709
Nostoc_17230725	LPASFIVGRYHSILFAQOPTIPGELKV TAISDENVMIMATEHOTLP IAAVQFHPPSIMTLAG	691
RhoPa_TpeG	LPNASFTVGRYHSILFADMPKLPVTATSDDCIAM IEHKTLIPAAVQFHPPSIMSLMG	702

Fig. 35D

1 ATGGTGACCA TCATTACAGGA TGACGCTGCC GAGACCTACG AGACCAAGGG CGGATCCAG
 61 GTGAGCCGCA AGCGCGCCC CACCGATTAC GCCAAGCCCA TCGATAACTA CATCGAAAAG
 121 CTTGATCCC ATCGCGGTG CGTGTTCTCC TCCAACTACG AATACCCAG CCGCTACACC
 181 CTTGCGGATA CGCGCATCGT CGATCCACCA CTCGGCATTT CTTGCTCGG CCGCAAGATG
 241 TGGATCGAAG CCTACAACGG CCGCGGGGAA GTGCTGCTCG ATTTTATTAC CGAAAGCTG
 301 AAGGCCACAC CCGATTCTAC CCTCGGCGT TCCTCCACC CCGCTCGA GTTACCGTC
 361 AACGACACG ACCGGTCTT CACCGAAGAA GAACGCTCCA AAATCCCAAC CGTCTTACC
 421 GCTCTCAGG CCATCGTCGA CCTTCTTAC TCCAGGCGG ATTCGCGCAT CGGCTGTTC
 481 GGTGCTTCG GTTACGATCT CGCCTTCCAG TTCGAGCCCA TCAAGCTTTC CCTGCGCCG
 541 CCAGAGACC AGCGGACAT GGTGCTGTT CCGCTGATG ATTCCTCGT CGTTGATCAC
 601 TACTCGGCC AGGCGTGGAT CGACGCTAC GATTTCGAGA AGGACGGCAT GACACCCAG
 661 GGCAATTCCT CCGACATTAC CCGCATCCC TTCAAGACCA CCGATACCAT CCGACCCAA
 721 GCGATACCC GCCCGGGCA ATACTCCGAG CTGTGTGTTA AGGCCAAGG AAGCTCCGC
 781 CGCGGCGACC TGTTCGAGGT CGTTCGCGC CAGAAATTCA TGGAGCGCTG CGAAAGCAAC
 841 CCATCGGCA TTTCGCGCG CCTGAGGCC ATCAACCCAT CCCCCTACTC CTTCCTCATC
 901 AACCTCGGG ATCAGGAATA CCTGTGCGG GCCTCCCCAG AATGTTCTG GCGCTCTCC
 961 GGCGCGGCA TCGAGACCTG CCCAATCTCA GGCACCATCA AGCGGCGGA CGATCCAAAT
 1021 GCGGACAGC AGCAGATTTT GAACTGCTC AACTCCAAA AGGACGAATC CGAACTGACC
 1081 ATGTGCTCG ACGTGGACCG CAACGACAA GCGCGGTCT GCGAGCCAGG TTCCTGAAG
 1141 GTCATGGCC GCGCCAGAT CGAGATGTAC TCACGCTCA TCCACACCGT CGATCACATC
 1201 GAAGCCGCC TGGCGACGA TATGACGCG CCTGACGCTT TCCTAGCCA CCGCTGGCC
 1261 GTACCGTCA CCGGTGCACC AAAGTGTGG GCCATCGCT TCATCGAAG TCATGAAAAG
 1321 AGCCACGCG CCTGTACGG CCGTGCCATC GGCATGGTC GCTTCAACGG CGACATGAAC
 1381 ACCGCGCTGA CCGTGGGAC CATCGGCAT AAGGACGGTA TTGCGAAGT GCGCGCGG
 1441 GCCACCTGC TCAACGATTC CAACCCACG GAAGAGGAAG CCGAACCAGA ACTGAAGCC
 1501 TCGGCGATGA TCTAGCCAT CCGGACGCA AAGGACCCA ACTTGGCCG CACCAAGCC
 1561 GATGCGGCCA AAGTCGGAC CGGCGTCAAG ATCTGCTCG TCGACCCAGA AGACAGCTTC

Fig. 36A

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1621 GTGCACACC TGGCAACTA CTTCCGCCAG ACGGCGCCA CCGTCTCCAC CGTCAGGTCA
1681 CCAGTCGCAG CCGACGTGT CGATCGCTTC CAGCCAGACC TCCTGTCTCT GTCCCCCGGT
1741 CCGGCGAGCC CAACGATTT CCACTGCAAG GCAACCATCA AGGCGGCCG CGCCGCGGAT
1801 CTGCCAATCT TCGCGTTTG CCTCGTCTG CAGGCATTGG CAGAAGCCTA CGGCGGCGAG
1861 CTGCGCCAGC TTGCTGTGCC CATGCACGGC AAGCCTTCCC GCATCGCGGT GCTGGAACCC
1921 GGCCTCGTCT TCTCCGGTCT CGGCAAGGAA GTCACCGTCG GTCGTACCA TTCCATCTTC
1981 GCCGATCCCG CCACCTGCC ACGCATTTC ATCATCACCG CAGAAGCGGA GGACGGCACC
2041 ATCATGGGCA TCGAACACGC CAAGNACCA GTGGCGGCCG TTCAGTTCCA CCCAGAATCC
2101 ATCATGACCC TCGGTGAGGA CGCCGGCATG CGCATGATCG AGAACGTCTG GGTGCATCTG
2161 ACCCGCAAGG CCAAGACCAA GGCCGGCTGA

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Fig. 36B

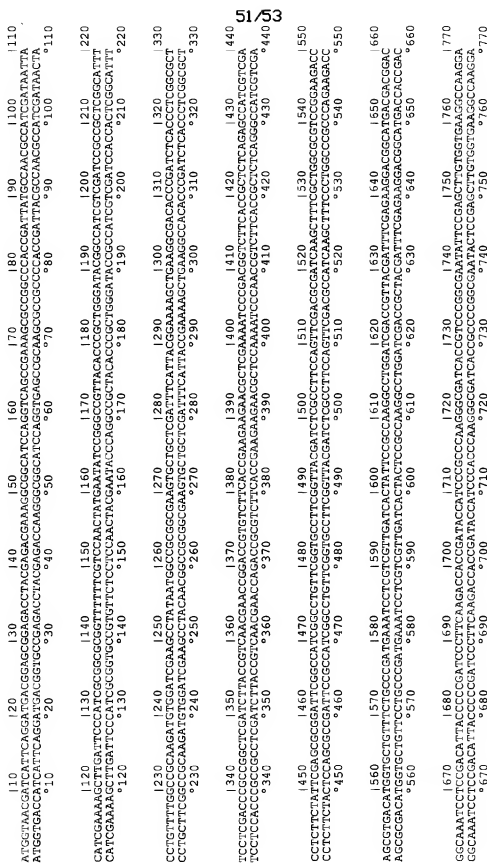


Fig. 37A

1780	1790	1800	1810	1820	1830	1840	1850	1860	1870	1880
AAGCTTCGCGCGCGGACCTGTTTCGAGGTCTGTTCCGCGCAGAAATTCATGGAGCGTTTCCAAAGCAATCCCTGCGGCAATTTCCCGCGCCTCGAGCGCATACACCGGT										
1780	1790	1800	1810	1820	1830	1840	1850	1860	1870	1880
AAGCTTCGCGCGCGGCGACCTGTTTCGAGGTCTGTTCCGCGCAGAAATTCATGGAGCGTTTCCAAAGCAATCCCTGCGGCAATTTCCCGCGCCTCGAGCGCATACACCGGT										
1890	1900	1910	1920	1930	1940	1950	1960	1970	1980	1990
CCCCCTACTCTCTTCTTCATCATATCTCGCGCATCAGGAATATCGTCTGGCGCTCGCGGCAATGTTTCTGCTCGCGCTCTCTCGCGCGCGCATCGAGACCTGCGCAATCTCA										
1890	1900	1910	1920	1930	1940	1950	1960	1970	1980	1990
CCCCCTACTCTCTTCTTCATCATATCTCGCGCATCAGGAATATCGTCTGGCGCTCGCGGCAATGTTTCTGCTCGCGCTCTCTCGCGCGCGCATCGAGACCTGCGCAATCTCA										
1000	1010	1020	1030	1040	1050	1060	1070	1080	1090	1100
GGCACCATCAAGCGCGGACGATCCCGATTCGCGACAGCAGAGATTTTGAATCTCTCAACTCCAAAAGGAGCAATCCGAATGCAATGTGCTCGGACGTGGACCG										
1000	1010	1020	1030	1040	1050	1060	1070	1080	1090	1100
GGCACCATCAAGCGCGGACGATCCCGATTCGCGACAGCAGAGATTTTGAATCTCTCAACTCCAAAAGGAGCAATCCGAATGCAATGTGCTCGGACGTGGACCG										
1110	1120	1130	1140	1150	1160	1170	1180	1190	1200	1210
CAACGACAAAGAGCGCGCTCGGAGCGCGGATTCGTTGAGGTGATTTGGCGCGCGCGAGATCGAGATGTATTTACAGCGCTCATCCACCGTCGATCACTCGAAGCGCGCC										
1110	1120	1130	1140	1150	1160	1170	1180	1190	1200	1210
CAACGACAAAGAGCGCGCTCGGAGCGCGGATTCGTTGAGGTGATTTGGCGCGCGCGAGATCGAGATGTATTTACAGCGCTCATCCACCGTCGATCACTCGAAGCGCGCC										
1220	1230	1240	1250	1260	1270	1280	1290	1300	1310	1320
TGCGCGACGATATGAGCGCTTTCTCAGCCAGCGCTGGCGGCTCACGTCACGCGTGCACAAAGCTGTGGCGCATCGCTTTCATCGAAGGTGATGAAG										
1220	1230	1240	1250	1260	1270	1280	1290	1300	1310	1320
TGCGCGACGATATGAGCGCTTTCTCAGCCAGCGCTGGCGGCTCACGTCACGCGTGCACAAAGCTGTGGCGCATCGCTTTCATCGAAGGTGATGAAG										
1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
AGCCCGCGCGCTTGATATGGCGGTGGGATCGGATGTGGCTTCAACGGCGACATGAATACCGGCTTGAGCTTGGCACCATTCGCGCATCAAGGACGGTATTCGCCAAGT										
1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
AGCCCGCGCGCTTGATATGGCGGTGGGATCGGATGTGGCTTCAACGGCGACATGAATACCGGCTTGAGCTTGGCACCATTCGCGCATCAAGGACGGTATTCGCCAAGT										
1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540
GGCGCGCGCGCTTGATATGGCGGTGGGATCGGATGTGGCTTCAACGGCGACATGAATACCGGCTTGAGCTTGGCACCATTCGCGCATCAAGGACGGTATTCGCCAAGT										
1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540
GGCGCGCGCGCTTGATATGGCGGTGGGATCGGATGTGGCTTCAACGGCGACATGAATACCGGCTTGAGCTTGGCACCATTCGCGCATCAAGGACGGTATTCGCCAAGT										

Fig. 37B

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Fig. 37C

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<110> Monsanto
 5 Renessen LLC
 Weaver, L.M.
 Liang, J.
 Chen, R.
 Jeong, S.S.
 10 Mitsky, T.
 Slater, S.
 Rapp, W.

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35 tcccttaaat	tctccaacgg	tctggtccca	cccagtcgcc	gtctgtctcc	gggtccgaac	180
aatgtcacct	gcaataacct	ccccagttct	gcagctcccg	tccggacagt	caaatgctgc	240
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	g c t c a t c c c g	t g a t g g a a g t	t a t a g c t a a a	g a t a a t a t c g	t t a c g g t g a t	g g a t c a t g a g	600
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	g a g g a t t g g a	a g c c t c a a a t	a a t g c a t g a t	c t t c c t g a a g	c t t t t t g c g g	t g g t t g g g t t	720
5	g g g t t t c t c t	c a t a c g a t a c	a g t c g a t a t	g t g g a g a a g a	a a a a g t t a c c	a t t c t c a a a g	780
	g c a c c t c a g g	a t g a t a g g a a	t c t t g c a g a t	a t g e a t c t a g	g t c t c t a t a a	c g a t g t t a t t	840
	g t g t t t g a t c	a t g t g g a a a a	g a a a g t a t a t	g t t a t t c a t t	g g g t g a g g c t	a a a t c a a c a g	900
	t c t t c t g a a g	a a a a g c a t c a	t g c c g a g g g t	c t g g a a c a c t	t g g a g a g a c t	a g t a t c c a a t	960
	g t a c g a g g a t	a g a a c a c g c c	a a g g g c t g c c	c c a g g t t c c a	t a g a c t t a c a	c a c t g g t c a t	1020
10	t t t g g a c c t c	c a t t a a a a a a	g c a a a c a t g a	a c a t g t g a a g	a a c a a a a a t	g g c t g t a c t a	1080
	g c g g c a a a a g	a a c a t a t t c a	g g c t g g g g a t	a t t t t t c a a a	t c g t a c t a a g	c c a a c g t t t t	1140
	g a a c g t c g a a	c a t t t g t c t a	t c c a t t t g a a	g t t t a t a g g g	c a c t g a g a g t	t g t t a a t c c g	1200
	a g t c c c t a t a	t g a c g t a t a t	g c a g g c a a g a	g g g t g t g t c t	t g g t a g c t c t	a a g t c c a g a a	1260
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	465		470		475	480
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	Ala	Val	Pro	Met	His	Gly
	625		630		635	640
	Gly	Leu	Val	Phe	Ser	Gly
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	His	Ser	Ile	Phe	Ala	Asp
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Ala Lys Ala Ala Glu Glu Asp Lys Arg Arg Phe Phe Glu Ala Ala Ala
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Arg Gly Ser Gly Lys Gly Asn Leu Val Pro Met Trp Glu Cys Ile Val
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Asp Asn Val Asp Ala Pro Ser Phe Leu Phe Glu Ser Val Glu Gln Gly
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Pro Val Met Glu Ile Val Ala Lys Asp His Lys Val Thr Ile Met Asp
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His Glu Lys Ser Gln Val Thr Glu Gln Val Val Asp Asp Pro Met Gln
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9

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Tyr Ser Val Val Gly Ala His Pro Val Met Glu Val Ile Ala Lys Asp
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Asn Met Val Thr Val Met Asp His Glu Lys Gly Ser Leu Val Glu Glu
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	His Leu Gly Leu Tyr Asn Asp Val Ile Val Phe Asp His Val Glu Lys				
	245	250	255		
	Lys Val Tyr Val Ile His Trp Val Arg Leu Asn Gln Gln Ser Ser Glu				
	260	265	270		
10	Glu Lys Ala Tyr Ala Glu Gly Leu Glu His Leu Glu Arg Leu Val Ser				
	275	280	285		
	Arg Val Gln Asp Glu Asn Thr Pro Arg Leu Ala Pro Gly Ser Ile Asp				
	290	295	300		
	Leu His Thr Gly His Phe Gly Pro Pro Leu Lys Lys Ser Asn Met Thr				
15	305	310	315	320	
	Cys Glu Glu Tyr Lys Met Ala Val Leu Ala Ala Lys Glu His Ile Gln				
	325	330	335		
	Ala Gly Asp Ile Phe Gln Ile Val Leu Ser Gln Arg Phe Glu Arg Arg				
	340	345	350		
20	Thr Phe Ala Asp Pro Phe Glu Val Tyr Arg Ala Leu Arg Val Val Asn				
	355	360	365		
	Pro Ser Pro Tyr Met Thr Tyr Met Gln Ala Arg Gly Cys Val Leu Val				
	370	375	380		
	Ala Ser Ser Pro Glu Ile Leu Thr Arg Val Lys Lys Asn Lys Ile Val				
25	385	390	395	400	
	Asn Arg Pro Leu Ala Gly Thr Ala Arg Arg Gly Arg Thr Thr Glu Glu				
	405	410	415		
	Asp Glu Met Leu Glu Thr Gln Leu Leu Lys Asp Ala Lys Gln Cys Ala				
	420	425	430		
30	Glu His Val Met Leu Val Asp Leu Gly Arg Asn Asp Val Gly Lys Val				
	435	440	445		
	Ser Lys Ser Gly Ser Val Lys Val Glu Lys Leu Met Asn Val Glu Arg				
	450	455	460		
	Tyr Ser His Val Met His Ile Ser Ser Thr Val Thr Gly Glu Leu Gln				
35	465	470	475	480	
	Asp Asn Leu Ser Cys Trp Asp Ala Leu Arg Ala Ala Leu Pro Val Gly				
	485	490	495		
	Thr Val Ser Gly Ala Pro Lys Val Lys Ala Met Glu Leu Ile Asp Glu				
	500	505	510		
40	Leu Glu Val Asn Arg Arg Gly Pro Tyr Ser Gly Gly Phe Gly Gly Ile				

11

515 520 525
 Ser Phe Thr Gly Asp Met Asp Ile Ala Leu Ala Leu Arg Thr Ile Val
 530 535 540
 Phe Gln Thr Gly Thr Arg Tyr Asp Thr Met Tyr Ser Tyr Lys Asn Ala
 5 545 550 555 560
 Thr Lys Arg Arg Gln Trp Val Ala Tyr Leu Gln Ala Gly Ala Gly Ile
 565 570 575
 Val Ala Asp Ser Asp Pro Asp Asp Glu His Arg Glu Cys Gln Asn Lys
 580 585 590
 10 Ala Ala Gly Leu Ala Arg Ala Ile Asp Leu Ala Glu Ser Ala Phe Val
 595 600 605
 Asn Lys Ser Ser Ser
 610

15 <210> 7

<211> 729

<212> PRT

<213> *Rhizobium meliloti*

20 <400> 7

Met Ala Ala Val Ile Leu Glu Asp Gly Ala Glu Ser Tyr Thr Thr Lys
 1 5 10 15
 Gly Gly Ile Val Val Thr Arg Arg Arg Arg Glu Ala Ser Tyr Ser Asp
 20 25 30
 25 Ala Ile Ala Gly Tyr Val Asp Arg Leu Asp Glu Arg Arg Gly Ala Val
 35 40 45
 Phe Ser Ser Asn Tyr Glu Tyr Pro Gly Arg Tyr Thr Arg Trp Asp Thr
 50 55 60
 Ala Val Val Asp Pro Pro Leu Ala Ile Ser Ser Phe Gly Arg Ser Leu
 30 65 70 75 80
 Trp Ile Glu Ala Tyr Asn Glu Arg Gly Glu Val Leu Leu Ala Leu Ile
 85 90 95
 Ala Glu Asp Leu Lys Ser Val Ala Asp Ile Thr Leu Gly Ser Leu Ala
 100 105 110
 35 Ala Arg Arg Leu Asp Leu Thr Ile Asn Glu Pro Asp Arg Val Phe Thr
 115 120 125
 Glu Glu Glu Arg Ser Lys Met Pro Thr Val Phe Thr Val Leu Arg Ala
 130 135 140
 Val Thr Asn Leu Phe His Ser Glu Glu Asp Ser Asn Leu Gly Leu Tyr
 40 145 150 155 160

12

Gly Ala Phe Gly Tyr Asp Leu Ala Phe Gln Phe Asp Ala Ile Glu Leu
 165 170 175
 Lys Leu Ser Arg Pro Asp Asp Gln Arg Asp Met Val Leu Phe Leu Pro
 180 185 190
 5 Asp Glu Ile Leu Val Val Asp His Tyr Ala Ala Lys Ala Trp Ile Asp
 195 200 205
 Arg Tyr Asp Phe Ala Arg Glu Asn Leu Ser Thr Glu Gly Lys Ala Ala
 210 215 220
 Asp Ile Ala Pro Glu Pro Phe Arg Ser Val Asp Ser Ile Pro Pro His
 10 225 230 235 240
 Gly Asp His Arg Pro Gly Glu Tyr Ala Glu Leu Val Val Lys Ala Lys
 245 250 255
 Glu Ser Phe Arg Arg Gly Asp Leu Phe Glu Val Val Pro Gly Gln Lys
 260 265 270
 15 Phe Tyr Glu Arg Cys Glu Ser Arg Pro Ser Glu Ile Ser Asn Arg Leu
 275 280 285
 Lys Ala Ile Asn Pro Ser Pro Tyr Ser Phe Phe Ile Asn Leu Gly Asn
 290 295 300
 Gln Glu Tyr Leu Val Gly Ala Ser Pro Glu Met Phe Val Arg Val Ser
 20 305 310 315 320
 Gly Arg Arg Ile Glu Thr Cys Pro Ile Ser Gly Thr Ile Lys Arg Gly
 325 330 335
 Asp Asp Pro Ile Ala Asp Ser Glu Gln Ile Leu Lys Leu Leu Asn Ser
 340 345 350
 25 Lys Lys Asp Glu Ser Glu Leu Thr Met Cys Ser Asp Val Asp Arg Asn
 355 360 365
 Asp Lys Ser Arg Val Cys Val Pro Gly Ser Val Lys Val Ile Gly Arg
 370 375 380
 Arg Gln Ile Glu Met Tyr Ser Arg Leu Ile His Thr Val Asp His Ile
 30 385 390 395 400
 Glu Gly Arg Leu Arg Asp Asp Met Asp Ala Phe Asp Gly Phe Leu Ser
 405 410 415
 His Ala Trp Ala Val Thr Val Thr Gly Ala Pro Lys Leu Trp Ala Met
 420 425 430
 35 Arg Phe Ile Glu Ser His Glu Lys Ser Pro Arg Ala Trp Tyr Gly Gly
 435 440 445
 Ala Ile Gly Met Val Gly Phe Asn Gly Asp Met Asn Thr Gly Leu Thr
 450 455 460
 Leu Arg Thr Ile Arg Ile Lys Asp Gly Ile Ala Glu Val Arg Ala Gly
 40 465 470 475 480

13

Ala Thr Leu Leu Tyr Asp Ser Asn Pro Glu Glu Glu Ala Glu Thr
 485 490 495

Glu Leu Lys Ala Ser Ala Met Ile Ala Ala Ile Arg Asp Ala Lys Ser
 500 505 510

5 Ala Asn Ser Ala Lys Ser Ala Arg Asp Val Ala Ala Val Gly Ala Gly
 515 520 525

Val Ser Ile Leu Leu Val Asp His Glu Asp Ser Phe Val His Thr Leu
 530 535 540

Ala Asn Tyr Phe Arg Gln Thr Gly Ala Ser Val Thr Thr Val Arg Thr
 10 545 550 555 560

Pro Val Ala Glu Glu Ile Phe Asp Arg Val Lys Pro Asp Leu Val Val
 565 570 575

Leu Ser Pro Gly Pro Gly Thr Pro Lys Asp Phe Asp Cys Lys Ala Thr
 580 585 590

15 Ile Lys Lys Ala Arg Ala Arg Asp Leu Pro Ile Phe Gly Val Cys Leu
 595 600 605

Gly Leu Gln Ala Leu Ala Glu Ala Tyr Gly Gly Asp Leu Arg Gln Leu
 610 615 620

Ala Ile Pro Met His Gly Lys Pro Ser Arg Ile Arg Val Leu Glu Pro
 20 625 630 635 640

Gly Ile Val Phe Ser Gly Leu Gly Lys Glu Val Thr Val Gly Arg Tyr
 645 650 655

His Ser Ile Phe Ala Asp Pro Ser Asn Leu Pro Arg Glu Phe Val Ile
 660 665 670

25 Thr Ala Glu Ser Glu Asp Gly Thr Ile Met Gly Ile Glu His Ser Lys
 675 680 685

Glu Pro Val Ala Ala Val Gln Phe His Pro Glu Ser Ile Met Thr Leu
 690 695 700

Gly Gly Asp Ala Gly Met Arg Met Ile Glu Asn Val Val Ala His Leu
 30 705 710 715 720

Ala Lys Arg Ala Lys Thr Lys Ala Ala
 725

<210> 8

35 <211> 421

<212> PRT

<213> *Sulfolobus solfataricus*

<400> 8

40 Met Glu Val His Pro Ile Ser Glu Phe Ala Ser Pro Phe Glu Val Phe

14

1	5	10	15													
Lys	Cys	Ile	Glu	Arg	Asp	Phe	Lys	Val	Ala	Gly	Leu	Leu	Glu	Ser	Ile	
				20					25					30		
	Gly	Gly	Pro	Gln	Tyr	Lys	Ala	Arg	Tyr	Ser	Val	Ile	Ala	Trp	Ser	Thr
5			35					40					45			
	Asn	Gly	Tyr	Leu	Lys	Ile	His	Asp	Asp	Pro	Val	Asn	Ile	Leu	Asn	Gly
			50				55					60				
	Tyr	Leu	Lys	Asp	Leu	Lys	Leu	Ala	Asp	Ile	Pro	Gly	Leu	Phe	Lys	Gly
65						70					75				80	
10	Gly	Met	Ile	Gly	Tyr	Ile	Ser	Tyr	Asp	Ala	Val	Arg	Phe	Trp	Glu	Lys
						85				90				95		
	Ile	Arg	Asp	Leu	Lys	Pro	Ala	Ala	Glu	Asp	Trp	Pro	Tyr	Ala	Glu	Phe
				100					105					110		
	Phe	Thr	Pro	Asp	Asn	Ile	Ile	Ile	Tyr	Asp	His	Asn	Glu	Gly	Lys	Val
15			115				120						125			
	Tyr	Val	Asn	Ala	Asp	Leu	Ser	Ser	Val	Gly	Gly	Cys	Gly	Asp	Ile	Gly
			130				135					140				
	Glu	Phe	Lys	Val	Ser	Phe	Tyr	Asp	Glu	Ser	Leu	Asn	Lys	Asn	Ser	Tyr
			145			150					155				160	
20	Glu	Arg	Ile	Val	Ser	Glu	Ser	Leu	Glu	Tyr	Ile	Arg	Ser	Gly	Tyr	Ile
				165						170				175		
	Phe	Gln	Val	Val	Leu	Ser	Arg	Phe	Tyr	Arg	Tyr	Ile	Phe	Ser	Gly	Asp
				180					185				190			
	Pro	Leu	Arg	Ile	Tyr	Tyr	Asn	Leu	Arg	Arg	Ile	Asn	Pro	Ser	Pro	Tyr
25			195				200						205			
	Met	Phe	Tyr	Leu	Lys	Phe	Asp	Glu	Lys	Tyr	Leu	Ile	Gly	Ser	Ser	Pro
			210			215					220					
	Glu	Leu	Leu	Phe	Arg	Val	Gln	Asp	Asn	Ile	Val	Glu	Thr	Tyr	Pro	Ile
225				230							235				240	
30	Ala	Gly	Thr	Arg	Pro	Arg	Gly	Ala	Asp	Gln	Glu	Glu	Asp	Leu	Lys	Leu
				245						250				255		
	Glu	Leu	Glu	Leu	Met	Asn	Ser	Glu	Lys	Asp	Lys	Ala	Glu	His	Leu	Met
				260					265				270			
	Leu	Val	Asp	Leu	Ala	Arg	Asn	Asp	Leu	Gly	Lys	Val	Cys	Val	Pro	Gly
35			275				280						285			
	Thr	Val	Lys	Val	Pro	Glu	Leu	Met	Tyr	Val	Glu	Lys	Tyr	Ser	His	Val
			290				295					300				
	Gln	His	Ile	Val	Ser	Lys	Val	Ile	Gly	Thr	Leu	Lys	Lys	Lys	Tyr	Asn
305				310							315				320	
40	Ala	Leu	Asn	Val	Leu	Ser	Ala	Thr	Phe	Pro	Ala	Gly	Thr	Val	Ser	Gly

15
 325 330 335
 Arg Pro Lys Pro Met Ala Met Asn Ile Ile Glu Thr Leu Glu Tyr
 340 345 350
 Lys Arg Gly Pro Tyr Ala Gly Ala Val Gly Phe Ile Ser Ala Asp Gly
 5 355 360 365
 Asn Ala Glu Phe Ala Ile Ala Ile Arg Thr Ala Phe Leu Asn Lys Glu
 370 375 380
 Leu Leu Arg Ile His Ala Gly Ala Gly Ile Val Tyr Asp Ser Asn Pro
 385 390 395 400
 10 Glu Ser Glu Tyr Phe Glu Thr Glu His Lys Leu Lys Ala Leu Lys Thr
 405 410 415
 Ala Ile Gly Val Arg
 420

15 <210> 9

<211> 32

<212> DNA

<213> Artificial Sequence

20 <220>

<223> A primer.

<400> 9

ccatcgcggc gcgttttttt cgtccaacta tg

32

25

<210> 10

<211> 32

<212> DNA

<213> Artificial Sequence

30

<220>

<223> A primer.

<400> 10

35 catagtggga cgaaaaaac gcgccgcgat gg

32

<210> 11

<211> 39

<212> DNA

40 <213> Artificial Sequence

<220>
<223> A primer.

5 <400> 11
ccatcgcggc gcgtatTTTT cgtccaacta tgaatatcc 39

<210> 12
<211> 39
10 <212> DNA
<213> Artificial Sequence

<220>
<223> A primer.

15
<400> 12
ggatattcat agttggacga aaaatacgcg cgcgatgg 39

<210> 13
20 <211> 39
<212> DNA
<213> Artificial Sequence

<220>
25 <223> A primer.

<400> 13
ccatcgcggc gcgtggtttt cgtccaacta tgaatatcc 39

30 <210> 14
<211> 39
<212> DNA
<213> Artificial Sequence

35 <220>
<223> A primer.

<400> 14
ggatattcat agttggacga aaaccacgcg cgcgatgg 39

40

<210> 15
<211> 39
<212> DNA
<213> Artificial Sequence
5
<220>
<223> A primer.

<400> 15
10 ccatacgcggc gcggttttta agtccaacta tgaatatcc 39

<210> 16
<211> 39
<212> DNA
15 <213> Artificial Sequence

<220>
<223> A primer.

20 <400> 16
ggatattcat agttggactt aaaaaccgcg ccgcgatgg 39

<210> 17
<211> 34
25 <212> DNA
<213> Artificial Sequence

<220>
<223> A primer.
30

<400> 17
gcgcgggtttt ttcgtgcaac tatgaatatc cggg 34

<210> 18
35 <211> 34
<212> DNA
<213> Artificial Sequence

<220>
40 <223> A primer.

<400> 18
cccggtatatt catagttgca cgaaaaaac gcgc 34

5 <210> 19
<211> 34
<212> DNA
<213> Artificial Sequence

10 <220>
<223> A primer.

<400> 19
cgcggttttt tcgttcaact atgaatatcc gggc 34

15
<210> 20
<211> 34
<212> DNA
<213> Artificial Sequence

20
<220>
<223> A primer.

<400> 20
25 gcccgatat tcatagttga acgaaaaaac cgcg 34

<210> 21
<211> 37
<212> DNA
30 <213> Artificial Sequence

<220>
<223> A primer.

35 <400> 21
cggcgcggtt ttttcgatca actatgaata tcggggc 37

<210> 22
<211> 37
40 <212> DNA

<213> Artificial Sequence

<220>

<223> A primer.

5

<400> 22
gcccgatat tcatagttga tcgaaaaaac cgcgccg 37

<210> 23

10 <211> 36

<212> DNA

<213> Artificial Sequence

<220>

15 <223> A primer.

<400> 23
ggcgcggttt ttctgctcaa ctatgaatat ccgggc 36

20 <210> 24

<211> 36

<212> DNA

<213> Artificial Sequence

25 <220>

<223> A primer.

<400> 24
gcccgatat tcatagttga gcgaaaaaac cgcgcc 36

30

<210> 25

<211> 39

<212> DNA

<213> Artificial Sequence

35

<220>

<223> A primer.

<400> 25
40 cggcgcggtt ttttcgatga actatgaata tccgggccg 39

<210> 26
<211> 39
<212> DNA
5 <213> Artificial Sequence

<220>
<223> A primer.

10 <400> 26
cggcccgcat attcatagtt catcgaaaaa accgogcgcg 39

<210> 27
<211> 34
15 <212> DNA
<213> Artificial Sequence

<220>
<223> A primer.

20
<400> 27
cgcggttttt tcgaccaact atgaatatcc gggc 34

<210> 28
25 <211> 34
<212> DNA
<213> Artificial Sequence

<220>
30 <223> A primer.

<400> 28
gcccgcatat tcatagttgg tcgaaaaaac cgcg 34

35 <210> 29
<211> 36
<212> DNA
<213> Artificial Sequence

40 <220>

21

<223> A primer.

<400> 29
ggcgcgggttt ttccggtcaa ctatgaatat cggggc 36

5

<210> 30
<211> 36
<212> DNA
<213> Artificial Sequence

10

<220>
<223> A primer.

<400> 30
15 gcccggtatat tcatagttga ccgaaaaaac cgcgcc 36

<210> 31
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<212> DNA
20 <213> Artificial Sequence

<220>
<223> A primer.

25 <400> 31
gcgcgggtttt ttcgtacaac tatgaatatc cggggc 35

<210> 32
<211> 35
30 <212> DNA
<213> Artificial Sequence

<220>
<223> A primer.

35

<400> 32
gcccggtatat tcatagttgt acgaaaaaac cgcgc 35

<210> 33
40 <211> 36

22

<212> DNA
<213> Artificial Sequence

<220>
5 <223> A primer.

<400> 33
cggcgcgggtt ttttcgtcct tctatgaata tcgggg 36

10 <210> 34
<211> 36
<212> DNA
<213> Artificial Sequence

15 <220>
<223> A primer.

<400> 34
cccggatatt catagaagga cgaaaaaacg gcgccg 36

20
<210> 35
<211> 29
<212> DNA
<213> Artificial Sequence

25
<220>
<223> A primer.

<400> 35
30 ctgaagcgga tcaacgcgtc gccctattc 29

<210> 36
<211> 29
<212> DNA
35 <213> Artificial Sequence

<220>
<223> A primer.

40 <400> 36

23

gaatagggcg acgcgttgat cgccttcag

29

<210> 37

<211> 31

5 <212> DNA

<213> Artificial Sequence

<220>

<223> A primer.

10

<400> 37

cctgaaggcg atcaacgggt cgccttattc c

31

<210> 38

15 <211> 31

<212> DNA

<213> Artificial Sequence

<220>

20 <223> A primer.

<400> 38

ggaatagggc gaccgcgttga tcgccttcag g

31

25 <210> 39

<211> 33

<212> DNA

<213> Artificial Sequence

30 <220>

<223> A primer.

<400> 39

cgtcgcccta ttccgccttc atcaatctcg gcg

33

35

<210> 40

<211> 33

<212> DNA

<213> Artificial Sequence

40

24

<220>

<223> A primer.

<400> 40

5 cgccgagatt gatgaaggcg gaatagggcg acg

33

<210> 41

<211> 33

<212> DNA

10 <213> Artificial Sequence

<220>

<223> A primer.

15 <400> 41

cgtcgcccta ttctcggttc atcaatctcg gcg

33

<210> 42

<211> 33

20 <212> DNA

<213> Artificial Sequence

<220>

<223> A primer.

25

<400> 42

cgccgagatt gatgaaccag gaatagggcg acg

33

<210> 43

30 <211> 729

<212> PRT

<213> Rhizobium meliloti

<400> 43

35 Met Ala Ala Val Ile Leu Glu Asp Gly Ala Glu Ser Tyr Thr Thr Lys

1

5

10

15

Gly Gly Ile Val Val Thr Arg Arg Arg Arg Glu Ala Ser Tyr Ser Asp

20

25

30

Ala Ile Ala Gly Tyr Val Asp Arg Leu Asp Glu Arg Arg Gly Ala Val

40

35

40

45

25

Phe Ser Ser Asn Tyr Glu Tyr Pro Gly Arg Tyr Thr Arg Trp Asp Thr
 50 55 60
 Ala Val Val Asp Pro Pro Leu Ala Ile Ser Ser Phe Gly Arg Ser Leu
 65 70 75 80
 5 Trp Ile Glu Ala Tyr Asn Glu Arg Gly Glu Val Leu Leu Ala Leu Ile
 85 90 95
 Ala Glu Asp Leu Lys Ser Val Ala Asp Ile Thr Leu Gly Ser Leu Ala
 100 105 110
 Ala Arg Arg Leu Asp Leu Thr Ile Asn Glu Pro Asp Arg Val Phe Thr
 10 115 120 125
 Glu Glu Glu Arg Ser Lys Met Pro Thr Val Phe Thr Val Leu Arg Ala
 130 135 140
 Val Thr Asn Leu Phe His Ser Glu Glu Asp Ser Asn Leu Gly Leu Tyr
 145 150 155 160
 15 Gly Ala Phe Gly Tyr Asp Leu Ala Phe Gln Phe Asp Ala Ile Glu Leu
 165 170 175
 Lys Leu Ser Arg Pro Asp Asp Gln Arg Asp Met Val Leu Phe Leu Pro
 180 185 190
 Asp Glu Ile Leu Val Val Asp His Tyr Ala Ala Lys Ala Trp Ile Asp
 20 195 200 205
 Arg Tyr Asp Phe Ala Arg Glu Asn Leu Ser Thr Glu Gly Lys Ala Ala
 210 215 220
 Asp Ile Ala Pro Glu Pro Phe Arg Ser Val Asp Ser Ile Pro Pro His
 225 230 235 240
 25 Gly Asp His Arg Pro Gly Glu Tyr Ala Glu Leu Val Val Lys Ala Lys
 245 250 255
 Glu Ser Phe Arg Arg Gly Asp Leu Phe Glu Val Val Pro Gly Gln Lys
 260 265 270
 Phe Tyr Glu Arg Cys Glu Ser Arg Pro Ser Glu Ile Ser Asn Arg Leu
 30 275 280 285
 Lys Ala Ile Asn Pro Ser Pro Tyr Ser Phe Phe Ile Asn Leu Gly Asn
 290 295 300
 Gln Glu Tyr Leu Val Gly Ala Ser Pro Glu Met Phe Val Arg Val Ser
 305 310 315 320
 35 Gly Arg Arg Ile Glu Thr Cys Pro Ile Ser Gly Thr Ile Lys Arg Gly
 325 330 335
 Asp Asp Pro Ile Ala Asp Ser Glu Gln Ile Leu Lys Leu Leu Asn Ser
 340 345 350
 Lys Lys Asp Glu Ser Glu Leu Thr Met Cys Ser Asp Val Asp Arg Asn
 40 355 360 365

26

Asp Lys Ser Arg Val Cys Val Pro Gly Ser Val Lys Val Ile Gly Arg
 370 375 380
 Arg Gln Ile Glu Met Tyr Ser Arg Leu Ile His Thr Val Asp His Ile
 385 390 395 400
 5 Glu Gly Arg Leu Arg Asp Asp Met Asp Ala Phe Asp Gly Phe Leu Ser
 405 410 415
 His Ala Trp Ala Val Thr Val Thr Gly Ala Pro Lys Leu Trp Ala Met
 420 425 430
 Arg Phe Ile Glu Ser His Glu Lys Ser Pro Arg Ala Trp Tyr Gly Gly
 10 435 440 445
 Ala Ile Gly Met Val Gly Phe Asn Gly Asp Met Asn Thr Gly Leu Thr
 450 455 460
 Leu Arg Thr Ile Arg Ile Lys Asp Gly Ile Ala Glu Val Arg Ala Gly
 465 470 475 480
 15 Ala Thr Leu Leu Tyr Asp Ser Asn Pro Glu Glu Glu Glu Ala Glu Thr
 485 490 495
 Glu Leu Lys Ala Ser Ala Met Ile Ala Ala Ile Arg Asp Ala Lys Ser
 500 505 510
 Ala Asn Ser Ala Lys Ser Ala Arg Asp Val Ala Ala Val Gly Ala Gly
 20 515 520 525
 Val Ser Ile Leu Leu Val Asp His Glu Asp Ser Phe Val His Thr Leu
 530 535 540
 Ala Asn Tyr Phe Arg Gln Thr Gly Ala Ser Val Thr Thr Val Arg Thr
 545 550 555 560
 25 Pro Val Ala Glu Glu Ile Phe Asp Arg Val Lys Pro Asp Leu Val Val
 565 570 575
 Leu Ser Pro Gly Pro Gly Thr Pro Lys Asp Phe Asp Cys Lys Ala Thr
 580 585 590
 Ile Lys Lys Ala Arg Ala Arg Asp Leu Pro Ile Phe Gly Val Cys Leu
 30 595 600 605
 Gly Leu Gln Ala Leu Ala Glu Ala Tyr Gly Gly Asp Leu Arg Gln Leu
 610 615 620
 Ala Ile Pro Met His Gly Lys Pro Ser Arg Ile Arg Val Leu Glu Pro
 625 630 635 640
 35 Gly Ile Val Phe Ser Gly Leu Gly Lys Glu Val Thr Val Gly Arg Tyr
 645 650 655
 His Ser Ile Phe Ala Asp Pro Ser Asn Leu Pro Arg Glu Phe Val Ile
 660 665 670
 Thr Ala Glu Ser Glu Asp Gly Thr Ile Met Gly Ile Glu His Ser Lys
 40 675 680 685

27

Glu Pro Val Ala Ala Val Gln Phe His Pro Glu Ser Ile Met Thr Leu
 690 695 700
 Gly Gly Asp Ala Gly Met Arg Met Ile Glu Asn Val Val Ala His Leu
 705 710 715 720
 5 Ala Lys Arg Ala Lys Thr Lys Ala Ala
 725

 <210> 44
 <211> 616
 10 <212> PRT
 <213> *Sulfolobus solfataricus*

 <400> 44
 Met Glu Val His Pro Ile Ser Glu Phe Ala Ser Pro Phe Glu Val Phe
 15 1 5 10 15
 Lys Cys Ile Glu Arg Asp Phe Lys Val Ala Gly Leu Leu Glu Ser Ile
 20 25 30
 Gly Gly Pro Gln Tyr Lys Ala Arg Tyr Ser Val Ile Ala Trp Ser Thr
 35 40 45
 20 Asn Gly Tyr Leu Lys Ile His Asp Asp Pro Val Asn Ile Leu Asn Gly
 50 55 60
 Tyr Leu Lys Asp Leu Lys Leu Ala Asp Ile Pro Gly Leu Phe Lys Gly
 65 70 75 80
 Gly Met Ile Gly Tyr Ile Ser Tyr Asp Ala Val Arg Phe Trp Glu Lys
 25 85 90 95
 Ile Arg Asp Leu Lys Pro Ala Ala Glu Asp Trp Pro Tyr Ala Glu Phe
 100 105 110
 Phe Thr Pro Asp Asn Ile Ile Ile Tyr Asp His Asn Glu Gly Lys Val
 115 120 125
 30 Tyr Val Asn Ala Asp Leu Ser Ser Val Gly Gly Cys Gly Asp Ile Gly
 130 135 140
 Glu Phe Lys Val Ser Phe Tyr Asp Glu Ser Leu Asn Lys Asn Ser Tyr
 145 150 155 160
 Glu Arg Ile Val Ser Glu Ser Leu Glu Tyr Ile Arg Ser Gly Tyr Ile
 35 165 170 175
 Phe Gln Val Val Leu Ser Arg Phe Tyr Arg Tyr Ile Phe Ser Gly Asp
 180 185 190
 Pro Leu Arg Ile Tyr Tyr Asn Leu Arg Arg Ile Asn Pro Ser Pro Tyr
 195 200 205
 40 Met Phe Tyr Leu Lys Phe Asp Glu Lys Tyr Leu Ile Gly Ser Ser Pro

28

	210		215		220	
	Glu Leu Leu Phe Arg Val Gln Asp Asn Ile Val Glu Thr Tyr Pro Ile					
	225		230		235	240
	Ala Gly Thr Arg Pro Arg Gly Ala Asp Gln Glu Glu Asp Leu Lys Leu					
5		245		250		255
	Glu Leu Glu Leu Met Asn Ser Glu Lys Asp Lys Ala Glu His Leu Met					
		260		265		270
	Leu Val Asp Leu Ala Arg Asn Asp Leu Gly Lys Val Cys Val Pro Gly					
	275		280		285	
10	Thr Val Lys Val Pro Glu Leu Met Tyr Val Glu Lys Tyr Ser His Val					
	290		295		300	
	Gln His Ile Val Ser Lys Val Ile Gly Thr Leu Lys Lys Lys Tyr Asn					
	305		310		315	320
	Ala Leu Asn Val Leu Ser Ala Thr Phe Pro Ala Gly Thr Val Ser Gly					
15		325		330		335
	Arg Pro Lys Pro Met Ala Met Asn Ile Ile Glu Thr Leu Glu Glu Tyr					
		340		345		350
	Lys Arg Gly Pro Tyr Ala Gly Ala Val Gly Phe Ile Ser Ala Asp Gly					
	355		360		365	
20	Asn Ala Glu Phe Ala Ile Ala Ile Arg Thr Ala Phe Leu Asn Lys Glu					
	370		375		380	
	Leu Leu Arg Ile His Ala Gly Ala Gly Ile Val Tyr Asp Ser Asn Pro					
	385		390		395	400
	Glu Ser Glu Tyr Phe Glu Thr Glu His Lys Leu Lys Ala Leu Lys Thr					
25		405		410		415
	Ala Ile Gly Val Arg Met Asp Leu Thr Leu Ile Ile Asp Asn Tyr Asp					
		420		425		430
	Ser Phe Val Tyr Asn Ile Ala Gln Ile Val Gly Glu Leu Gly Ser Tyr					
	435		440		445	
30	Pro Ile Val Ile Arg Asn Asp Glu Ile Ser Ile Lys Gly Ile Glu Arg					
	450		455		460	
	Ile Asp Pro Asp Arg Leu Ile Ile Ser Pro Gly Pro Gly Thr Pro Glu					
	465		470		475	480
	Lys Arg Glu Asp Ile Gly Val Ser Leu Asp Val Ile Lys Tyr Leu Gly					
35		485		490		495
	Lys Arg Thr Pro Ile Leu Gly Val Cys Leu Gly His Gln Ala Ile Gly					
		500		505		510
	Tyr Ala Phe Gly Ala Lys Ile Arg Arg Ala Arg Lys Val Phe His Gly					
	515		520		525	
40	Lys Ile Ser Asn Ile Ile Leu Val Asn Asn Ser Pro Leu Ser Leu Tyr					

29

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      530              535              540
Tyr Gly Ile Ala Lys Glu Phe Lys Ala Thr Arg Tyr His Ser Leu Val
545              550              555              560
Val Asp Glu Val His Arg Pro Leu Ile Val Asp Ala Ile Ser Ala Glu
5      565              570              575
Asp Asn Glu Ile Met Ala Ile His His Glu Glu Tyr Pro Ile Tyr Gly
      580              585              590
Val Gln Phe His Pro Glu Ser Val Gly Thr Ser Leu Gly Tyr Lys Ile
      595              600              605
10 Leu Tyr Asn Phe Leu Asn Arg Val
      610              615

<210> 45
<211> 897
15 <212> PRT
    <213> Arabidopsis thaliana

<400> 45
Met Ser Ala Val Ser Ile Ser Ala Val Lys Ser Asp Phe Phe Thr Val
20 1              5              10              15
Glu Ala Ile Ala Val Thr His His Arg Thr Pro His Pro Pro His Phe
      20              25              30
Pro Ser Leu Arg Phe Pro Leu Ser Leu Lys Ser Pro Pro Ala Thr Ser
      35              40              45
25 Leu Asn Leu Val Ala Gly Ser Lys Leu Leu His Phe Ser Arg Arg Leu
      50              55              60
Pro Ser Ile Lys Cys Ser Tyr Thr Pro Ser Leu Asp Leu Ser Glu Glu
65              70              75              80
Gln Phe Thr Lys Phe Lys Lys Ala Ser Glu Lys Gly Asn Leu Val Pro
30      85              90              95
Leu Phe Arg Cys Val Phe Ser Asp His Leu Thr Pro Ile Leu Ala Tyr
      100              105              110
Arg Cys Leu Val Lys Glu Asp Asp Arg Asp Ala Pro Ser Phe Leu Phe
      115              120              125
35 Glu Ser Val Glu Pro Gly Ser Gln Ser Ser Asn Ile Gly Arg Tyr Ser
      130              135              140
Val Val Gly Ala Gln Pro Thr Ile Glu Ile Val Ala Lys Gly Asn Val
145              150              155              160
Val Thr Val Met Asp His Gly Ala Ser Leu Arg Thr Glu Glu Glu Val
40      165              170              175

```

30

Asp Asp Pro Met Met Val Pro Gln Lys Ile Met Glu Glu Trp Asn Pro
 180 185 190
 Gln Gly Ile Asp Glu Leu Pro Glu Ala Phe Cys Gly Gly Trp Val Gly
 195 200 205
 5 Tyr Phe Ser Tyr Asp Thr Val Arg Tyr Val Glu Lys Lys Lys Leu Pro
 210 215 220
 Phe Ser Asn Ala Pro Glu Asp Asp Arg Ser Leu Pro Asp Val Asn Leu
 225 230 235 240
 Gly Leu Tyr Asp Asp Val Ile Val Phe Asp His Val Glu Lys Lys Ala
 10 245 250 255
 Tyr Val Ile His Trp Val Arg Ile Asp Lys Asp Arg Ser Val Glu Glu
 260 265 270
 Asn Phe Arg Glu Gly Met Asn Arg Leu Glu Ser Leu Thr Ser Arg Ile
 275 280 285
 15 Gln Asp Gln Lys Pro Pro Lys Met Pro Thr Gly Phe Ile Lys Leu Arg
 290 295 300
 Thr Gln Leu Phe Gly Pro Lys Leu Glu Lys Ser Thr Met Thr Ser Glu
 305 310 315 320
 Ala Tyr Lys Glu Ala Val Val Glu Ala Lys Glu His Ile Leu Ala Gly
 20 325 330 335
 Asp Ile Phe Gln Ile Val Leu Ser Gln Arg Phe Glu Arg Arg Thr Phe
 340 345 350
 Ala Asp Pro Phe Glu Ile Tyr Arg Ala Leu Arg Ile Val Asn Pro Ser
 355 360 365
 25 Pro Tyr Met Ala Tyr Leu Gln Val Arg Gly Cys Ile Leu Val Ala Ser
 370 375 380
 Ser Pro Glu Ile Leu Leu Arg Ser Lys Asn Arg Lys Ile Thr Asn Arg
 385 390 395 400
 Pro Leu Ala Gly Thr Val Arg Arg Gly Lys Thr Pro Lys Glu Asp Leu
 30 405 410 415
 Met Leu Glu Lys Glu Leu Leu Ser Asp Glu Lys Gln Cys Ala Glu His
 420 425 430
 Ile Met Leu Val Asp Leu Gly Arg Asn Asp Val Gly Lys Val Ser Lys
 435 440 445
 35 Pro Gly Ser Val Glu Val Lys Lys Leu Lys Asp Ile Glu Trp Phe Ser
 450 455 460
 His Val Met His Ile Ser Ser Thr Val Val Gly Glu Leu Leu Asp His
 465 470 475 480
 Leu Thr Ser Trp Asp Ala Leu Arg Ala Val Leu Pro Val Gly Thr Val
 40 485 490 495

31

Ser Gly Ala Pro Lys Val Lys Ala Met Glu Leu Ile Asp Glu Leu Glu
 500 505 510
 Val Thr Arg Arg Gly Pro Tyr Ser Gly Gly Phe Gly Gly Ile Ser Phe
 515 520 525
 5 Asn Gly Asp Met Asp Ile Ala Leu Ala Leu Arg Thr Met Val Phe Pro
 530 535 540
 Thr Asn Thr Arg Tyr Asp Thr Leu Tyr Ser Tyr Lys His Pro Gln Arg
 545 550 555 560
 Arg Arg Glu Trp Ile Ala His Ile Gln Ala Gly Ala Gly Ile Val Ala
 10 565 570 575
 Asp Ser Asn Pro Asp Asp Glu His Arg Glu Cys Glu Asn Lys Ala Ala
 580 585 590
 Ala Leu Ala Arg Ala Ile Asp Leu Ala Glu Ser Ser Phe Leu Glu Ala
 595 600 605
 15 Pro Glu Phe Thr Thr Ile Thr Pro His Ile Asn Asn Ile Met Ala Ala
 610 615 620
 Ser Thr Leu Tyr Lys Ser Cys Leu Leu Gln Pro Lys Ser Gly Ser Thr
 625 630 635 640
 Thr Arg Arg Leu Asn Pro Ser Leu Val Asn Pro Leu Thr Asn Pro Thr
 20 645 650 655
 Arg Val Ser Val Leu Gly Lys Ser Arg Arg Asp Val Phe Ala Lys Ala
 660 665 670
 Ser Ile Glu Met Ala Glu Ser Asn Ser Ile Pro Ser Val Val Val Asn
 675 680 685
 25 Ser Ser Lys Gln His Gly Pro Ile Ile Val Ile Asp Asn Tyr Asp Ser
 690 695 700
 Phe Thr Tyr Asn Leu Cys Gln Tyr Met Gly Glu Leu Gly Cys His Phe
 705 710 715 720
 Glu Val Tyr Arg Asn Asp Glu Leu Thr Val Glu Glu Leu Lys Lys Lys
 30 725 730 735
 Asn Pro Arg Gly Val Leu Ile Ser Pro Gly Pro Gly Thr Pro Gln Asp
 740 745 750
 Ser Gly Ile Ser Leu Gln Thr Val Leu Glu Leu Gly Pro Leu Val Pro
 755 760 765
 35 Leu Phe Gly Val Cys Met Gly Leu Gln Cys Ile Gly Glu Ala Phe Gly
 770 775 780
 Gly Lys Ile Val Arg Ser Pro Phe Gly Val Met His Gly Lys Ser Ser
 785 790 795 800
 Met Val His Tyr Asp Glu Lys Gly Glu Glu Gly Leu Phe Ser Gly Leu
 40 805 810 815

32

Ser Asn Pro Phe Ile Val Gly Arg Tyr His Ser Leu Val Ile Glu Lys
 820 825 830
 Asp Thr Phe Pro Ser Asp Glu Leu Glu Val Thr Ala Trp Thr Glu Asp
 835 840 845
 5 Gly Leu Val Met Ala Ala Arg His Arg Lys Tyr Lys His Ile Gln Gly
 850 855 860
 Val Gln Phe His Pro Glu Ser Ile Ile Thr Thr Glu Gly Lys Thr Ile
 865 870 875 880
 Val Arg Asn Phe Ile Lys Ile Val Glu Lys Lys Glu Ser Glu Lys Leu
 10 885 890 895
 Thr

<210> 46

15 <211> 252

<212> DNA

<213> Artificial Sequence

<220>

20 <223> A truncated gene

<400> 46

atgcaaacac aaaaaccgac tctcgaaactg gaattcctgg tggaaaacgg tatcgccacc 60
 gtgcaagcgg gtgctggtgt agtccttgat tctgttcgcg agtcggaagc cgacgaaacc 120
 25 cgtaacaaag cccgcgctgt actgcgcgct attgccaccg cgcacatgc acaggagact 180
 ttctgatggc tgacattctg ctgctcgata atatcgactc ttttacgtac aacctggcag 240
 atcagttgcg ca 252

<210> 47

30 <211> 18

<212> DNA

<213> Artificial Sequence

<220>

35 <223> A primer.

<400> 47

ttatgccgcc tgtcatcg 18

40 <210> 48

<211> 19
<212> DNA
<213> Artificial Sequence

5 <220>
<223> A primer.

<400> 48
ataggcttaa tggtaaccg 19

10
<210> 49
<211> 18
<212> DNA
<213> Artificial Sequence

15
<220>
<223> A primer.

<400> 49
20 ctgaacaaca gaagtacg 18

<210> 50
<211> 18
<212> DNA
25 <213> Artificial Sequence

<220>
<223> A primer.

30 <400> 50
taaccgtgac atcgagcg 18

<210> 51
<211> 31
35 <212> DNA
<213> Artificial Sequence

<220>
<223> A primer

40

34

<400> 51
aaaaagatct ccatggtaac gatcattcag g 31

<210> 52
5 <211> 35
<212> DNA
<213> Artificial Sequence

<220>
10 <223> A primer

<400> 52
aaaagaattc ttatcacgcg gccttggtct tcgcc 35

15 <210> 53
<211> 19
<212> DNA
<213> Artificial Sequence

20 <220>
<223> A primer

<400> 53
caaaagctgg atccccacc 19

25
<210> 54
<211> 23
<212> DNA
<213> Artificial Sequence

30
<220>
<223> A primer

<400> 54
35 cctatccgag atctctcaac tcc 23

<210> 55
<211> 31
<212> DNA
40 <213> Artificial Sequence

35

<220>
 <223> A primer

5 <400> 55
 catcccatgg atggtaacga tcattcagga t 31

<210> 56
 <211> 31
 10 <212> DNA
 <213> Artificial Sequence

<220>
 <223> A primer

15
 <400> 56
 gatgtctaga gacactatag aatactcaag c 31

<210> 57
 20 <211> 719
 <212> PRT
 <213> *Rhodopseudomonas palustris*

<400> 57

25 Met Asn Arg Thr Val Phe Ser Leu Pro Ala Thr Ser Asp Tyr Lys Thr
 1 5 10 15
 Ala Ala Gly Leu Ala Val Thr Arg Ser Ala Gln Pro Phe Ala Gly Gly
 20 25 30
 Gln Ala Leu Asp Glu Leu Ile Asp Leu Leu Asp His Arg Arg Gly Val
 30 35 40 45
 Met Leu Ser Ser Gly Thr Thr Val Pro Gly Arg Tyr Glu Ser Phe Asp
 50 55 60
 Leu Gly Phe Ala Asp Pro Leu Ala Leu Thr Thr Arg Ala Glu Lys
 65 70 75 80
 35 Phe Thr Ile Glu Ala Leu Asn Pro Arg Gly Arg Val Leu Ile Ala Phe
 85 90 95
 Leu Ser Asp Lys Leu Glu Glu Pro Cys Val Val Val Glu Gln Ala Cys
 100 105 110
 Ala Thr Lys Ile Arg Gly His Ile Val Arg Gly Glu Ala Pro Val Asp
 140 115 120 125

36

Glu Glu Gln Arg Thr Arg Arg Ala Ser Ala Ile Ser Leu Val Arg Ala
 130 135 140
 Val Ile Ala Ala Phe Ala Ser Pro Ala Asp Pro Met Leu Gly Leu Tyr
 145 150 155 160
 5 Gly Ala Phe Ala Tyr Asp Leu Val Phe Gln Phe Glu Asp Leu Lys Gln
 165 170 175
 Lys Arg Ala Arg Glu Ala Asp Gln Arg Asp Ile Val Leu Tyr Val Pro
 180 185 190
 Asp Arg Leu Leu Ala Tyr Asp Arg Ala Thr Gly Arg Gly Val Asp Ile
 10 195 200 205
 Ser Tyr Glu Phe Ala Trp Lys Gly Gln Ser Thr Ala Gly Leu Pro Asn
 210 215 220
 Glu Thr Ala Glu Ser Val Tyr Thr Gln Thr Gly Arg Gln Gly Phe Ala
 225 230 235 240
 15 Asp His Ala Pro Gly Asp Tyr Pro Lys Val Val Glu Lys Ala Arg Ala
 245 250 255
 Ala Phe Ala Arg Gly Asp Leu Phe Glu Ala Val Pro Gly Gln Leu Phe
 260 265 270
 Gly Glu Pro Cys Glu Arg Ser Pro Ala Glu Val Phe Lys Arg Leu Cys
 20 275 280 285
 Arg Ile Asn Pro Ser Pro Tyr Gly Gly Leu Leu Asn Leu Gly Asp Gly
 290 295 300
 Glu Phe Leu Val Ser Ala Ser Pro Glu Met Phe Val Arg Ser Asp Gly
 305 310 315 320
 25 Arg Arg Ile Glu Thr Cys Pro Ile Ser Gly Thr Ile Ala Arg Gly Val
 325 330 335
 Asp Ala Ile Ser Asp Ala Glu Gln Ile Gln Lys Leu Leu Asn Ser Glu
 340 345 350
 Lys Asp Glu Phe Glu Leu Asn Met Cys Thr Asp Val Asp Arg Asn Asp
 30 355 360 365
 Lys Ala Arg Val Cys Val Pro Gly Thr Ile Lys Val Leu Ala Arg Arg
 370 375 380
 Gln Ile Glu Thr Tyr Ser Lys Leu Phe His Thr Val Asp His Val Glu
 385 390 395 400
 35 Gly Met Leu Arg Pro Gly Phe Asp Ala Leu Asp Ala Phe Leu Thr His
 405 410 415
 Ala Trp Ala Val Thr Val Thr Gly Ala Pro Lys Leu Trp Ala Met Gln
 420 425 430
 Phe Val Glu Asp His Glu Arg Ser Pro Arg Arg Trp Tyr Ala Gly Ala
 40 435 440 445

37

Phe Gly Val Val Gly Phe Asp Gly Ser Ile Asn Thr Gly Leu Thr Ile
 450 455 460
 Arg Thr Ile Arg Met Lys Asp Gly Leu Ala Glu Val Arg Val Gly Ala
 465 470 475 480
 5 Thr Cys Leu Phe Asp Ser Asn Pro Val Ala Glu Asp Lys Glu Cys Gln
 485 490 495
 Val Lys Ala Ala Leu Phe Gln Ala Leu Arg Gly Asp Pro Ala Lys
 500 505 510
 Pro Leu Ser Ala Val Ala Pro Asp Ala Thr Gly Ser Gly Lys Lys Val
 10 515 520 525
 Leu Leu Val Asp His Asp Asp Ser Phe Val His Met Leu Ala Asp Tyr
 530 535 540
 Phe Arg Gln Val Gly Ala Gln Val Thr Val Val Arg Tyr Val His Gly
 545 550 555 560
 15 Leu Lys Met Leu Ala Glu Asn Ser Tyr Asp Leu Leu Val Leu Ser Pro
 565 570 575
 Gly Pro Gly Arg Pro Glu Asp Phe Lys Ile Lys Asp Thr Ile Asp Ala
 580 585 590
 Ala Leu Ala Lys Lys Leu Pro Ile Phe Gly Val Cys Leu Gly Val Gln
 20 595 600 605
 Ala Met Gly Glu Tyr Phe Gly Gly Thr Leu Gly Gln Leu Ala Gln Pro
 610 615 620
 Ala His Gly Arg Pro Ser Arg Ile Gln Val Arg Gly Gly Ala Leu Met
 625 630 635 640
 25 Arg Gly Leu Pro Asn Glu Val Thr Ile Gly Arg Tyr His Ser Leu Tyr
 645 650 655
 Val Asp Met Arg Asp Met Pro Lys Glu Leu Thr Val Thr Ala Ser Thr
 660 665 670
 Asp Asp Gly Ile Ala Met Ala Ile Glu His Lys Thr Leu Pro Val Gly
 30 675 680 685
 Gly Val Gln Phe His Pro Glu Ser Leu Met Ser Leu Gly Gly Glu Val
 690 695 700
 Gly Leu Arg Ile Val Glu Asn Ala Phe Arg Leu Gly Gln Ala Ala
 705 710 715
 35
 <210> 58
 <211> 729
 <212> PRT
 <213> Artificial Sequence

<220>

<223> An *A. tumefaciens* mutant.

<400> 58

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5 Met Val Thr Ile Ile Gln Asp Asp Gly Ala Glu Thr Tyr Glu Thr Lys
  1           5           10           15
  Gly Gly Ile Gln Val Ser Arg Lys Arg Arg Pro Thr Asp Tyr Ala Asn
    20           25           30
  Ala Ile Asp Asn Tyr Ile Glu Lys Leu Asp Ser His Arg Gly Ala Phe
10   35           40           45
  Phe Ser Ser Asn Tyr Glu Tyr Pro Gly Arg Tyr Thr Arg Trp Asp Thr
    50           55           60
  Ala Ile Val Asp Pro Pro Leu Gly Ile Ser Cys Phe Gly Arg Lys Met
  65           70           75           80
15 Trp Ile Glu Ala Tyr Asn Gly Arg Gly Glu Val Leu Leu Asp Phe Ile
    85           90           95
  Thr Glu Lys Leu Lys Ala Thr Pro Asp Leu Thr Leu Gly Ala Ser Ser
    100          105          110
  Thr Arg Arg Leu Asp Leu Thr Val Asn Glu Pro Asp Arg Val Phe Thr
20   115          120          125
  Glu Glu Glu Arg Ser Lys Ile Pro Thr Val Phe Thr Ala Leu Arg Ala
    130          135          140
  Ile Val Asp Leu Phe Tyr Ser Ser Ala Asp Ser Ala Ile Gly Leu Phe
  145          150          155          160
25 Gly Ala Phe Gly Tyr Asp Leu Ala Phe Gln Phe Asp Ala Ile Lys Leu
    165          170          175
  Ser Leu Ala Arg Pro Glu Asp Gln Arg Asp Met Val Leu Phe Leu Pro
    180          185          190
  Asp Glu Ile Leu Val Val Asp His Tyr Ser Ala Lys Ala Trp Ile Asp
30   195          200          205
  Arg Tyr Asp Phe Glu Lys Asp Gly Met Thr Thr Asp Gly Lys Ser Ser
    210          215          220
  Asp Ile Thr Pro Asp Pro Phe Lys Thr Thr Asp Thr Ile Pro Pro Lys
  225          230          235          240
35 Gly Asp His Arg Pro Gly Glu Tyr Ser Glu Leu Val Val Lys Ala Lys
    245          250          255
  Glu Ser Phe Arg Arg Gly Asp Leu Phe Glu Val Val Pro Gly Gln Lys
    260          265          270
  Phe Met Glu Arg Cys Glu Ser Asn Pro Ser Ala Ile Ser Arg Arg Leu
40   275          280          285

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39

Lys Ala Ile Asn Pro Ser Pro Tyr Ser Phe Phe Ile Asn Leu Gly Asp
 290 295 300
 Gln Glu Tyr Leu Val Gly Ala Ser Pro Glu Met Phe Val Arg Val Ser
 305 310 315 320
 5 Gly Arg Arg Ile Glu Thr Cys Pro Ile Ser Gly Thr Ile Lys Arg Gly
 325 330 335
 Asp Asp Pro Ile Ala Asp Ser Glu Gln Ile Leu Lys Leu Leu Asn Ser
 340 345 350
 Lys Lys Asp Glu Ser Glu Leu Thr Met Cys Ser Asp Val Asp Arg Asn
 10 355 360 365
 Asp Lys Ser Arg Val Cys Glu Pro Gly Ser Val Lys Val Ile Gly Arg
 370 375 380
 Arg Gln Ile Glu Met Tyr Ser Arg Leu Ile His Thr Val Asp His Ile
 385 390 395 400
 15 Glu Gly Arg Leu Arg Asp Asp Met Asp Ala Phe Asp Gly Phe Leu Ser
 405 410 415
 His Ala Trp Ala Val Thr Val Thr Gly Ala Pro Lys Leu Trp Ala Met
 420 425 430
 Arg Phe Ile Glu Gly His Glu Lys Ser Pro Arg Ala Trp Tyr Gly Gly
 20 435 440 445
 Ala Ile Gly Met Val Gly Phe Asn Gly Asp Met Asn Thr Gly Leu Thr
 450 455 460
 Leu Arg Thr Ile Arg Ile Lys Asp Gly Ile Ala Glu Val Arg Ala Gly
 465 470 475 480
 25 Ala Thr Leu Leu Asn Asp Ser Asn Pro Gln Glu Glu Glu Ala Glu Thr
 485 490 495
 Glu Leu Lys Ala Ser Ala Met Ile Ser Ala Ile Arg Asp Ala Lys Gly
 500 505 510
 Thr Asn Ser Ala Ala Thr Lys Arg Asp Ala Ala Lys Val Gly Thr Gly
 30 515 520 525
 Val Lys Ile Leu Leu Val Asp His Glu Asp Ser Phe Val His Thr Leu
 530 535 540
 Ala Asn Tyr Phe Arg Gln Thr Gly Ala Thr Val Ser Thr Val Arg Ser
 545 550 555 560
 35 Pro Val Ala Ala Asp Val Phe Asp Arg Phe Gln Pro Asp Leu Val Val
 565 570 575
 Leu Ser Pro Gly Pro Gly Ser Pro Thr Asp Phe Asp Cys Lys Ala Thr
 580 585 590
 Ile Lys Ala Ala Arg Ala Arg Asp Leu Pro Ile Phe Gly Val Cys Leu
 40 595 600 605

40

Gly Leu Gln Ala Leu Ala Glu Ala Tyr Gly Gly Glu Leu Arg Gln Leu
 610 615 620
 Ala Val Pro Met His Gly Lys Pro Ser Arg Ile Arg Val Leu Glu Pro
 625 630 635 640
 5 Gly Leu Val Phe Ser Gly Leu Gly Lys Glu Val Thr Val Gly Arg Tyr
 645 650 655
 His Ser Ile Phe Ala Asp Pro Ala Thr Leu Pro Arg Asp Phe Ile Ile
 660 665 670
 Thr Ala Glu Ser Glu Asp Gly Thr Ile Met Gly Ile Glu His Ala Lys
 10 675 680 685
 Glu Pro Val Ala Ala Val Gln Phe His Pro Glu Ser Ile Met Thr Leu
 690 695 700
 Gly Gln Asp Ala Gly Met Arg Met Ile Glu Asn Val Val His Leu
 705 710 715 720
 15 Thr Arg Lys Ala Lys Thr Lys Ala Ala
 725

<210> 59

<211> 729

20 <212> PRT

<213> Artificial Sequence

<220>

<223> An A. tumefaciens mutant.

25

<400> 59

Met Val Thr Ile Ile Gln Asp Asp Gly Ala Glu Thr Tyr Glu Thr Lys
 1 5 10 15
 Gly Gly Ile Gln Val Ser Arg Lys Arg Arg Pro Thr Asp Tyr Ala Asn
 30 20 25 30
 Ala Ile Asp Asn Tyr Ile Glu Lys Leu Asp Ser His Arg Gly Ala Tyr
 35 40 45
 Phe Ser Ser Asn Tyr Glu Tyr Pro Gly Arg Tyr Thr Arg Trp Asp Thr
 50 55 60
 35 Ala Ile Val Asp Pro Pro Leu Gly Ile Ser Cys Phe Gly Arg Lys Met
 65 70 75 80
 Trp Ile Glu Ala Tyr Asn Gly Arg Gly Glu Val Leu Leu Asp Phe Ile
 85 90 95
 Thr Glu Lys Leu Lys Ala Thr Pro Asp Leu Thr Leu Gly Ala Ser Ser
 40 100 105 110

41

Thr	Arg	Arg	Leu	Asp	Leu	Thr	Val	Asn	Glu	Pro	Asp	Arg	Val	Phe	Thr
	115						120				125				
Glu	Glu	Glu	Arg	Ser	Lys	Ile	Pro	Thr	Val	Phe	Thr	Ala	Leu	Arg	Ala
	130					135					140				
5	Ile	Val	Asp	Leu	Phe	Tyr	Ser	Ser	Ala	Asp	Ser	Ala	Ile	Gly	Leu
	145					150				155					160
Gly	Ala	Phe	Gly	Tyr	Asp	Leu	Ala	Phe	Gln	Phe	Asp	Ala	Ile	Lys	Leu
					165				170					175	
Ser	Leu	Ala	Arg	Pro	Glu	Asp	Gln	Arg	Asp	Met	Val	Leu	Phe	Leu	Pro
10			180						185				190		
Asp	Glu	Ile	Leu	Val	Val	Asp	His	Tyr	Ser	Ala	Lys	Ala	Trp	Ile	Asp
			195					200				205			
Arg	Tyr	Asp	Phe	Glu	Lys	Asp	Gly	Met	Thr	Thr	Asp	Gly	Lys	Ser	Ser
	210					215					220				
15	Asp	Ile	Thr	Pro	Asp	Pro	Phe	Lys	Thr	Thr	Asp	Thr	Ile	Pro	Pro
	225					230					235			240	
Gly	Asp	His	Arg	Pro	Gly	Glu	Tyr	Ser	Glu	Leu	Val	Val	Lys	Ala	Lys
					245				250					255	
Glu	Ser	Phe	Arg	Arg	Gly	Asp	Leu	Phe	Glu	Val	Val	Pro	Gly	Gln	Lys
20			260					265				270			
Phe	Met	Glu	Arg	Cys	Glu	Ser	Asn	Pro	Ser	Ala	Ile	Ser	Arg	Arg	Leu
			275				280				285				
Lys	Ala	Ile	Asn	Pro	Ser	Pro	Tyr	Ser	Phe	Phe	Ile	Asn	Leu	Gly	Asp
	290					295				300					
25	Gln	Glu	Tyr	Leu	Val	Gly	Ala	Ser	Pro	Glu	Met	Phe	Val	Arg	Val
	305					310					315				320
Gly	Arg	Arg	Ile	Glu	Thr	Cys	Pro	Ile	Ser	Gly	Thr	Ile	Lys	Arg	Gly
			325						330					335	
Asp	Asp	Pro	Ile	Ala	Asp	Ser	Glu	Gln	Ile	Leu	Lys	Leu	Leu	Asn	Ser
30			340					345				350			
Lys	Lys	Asp	Glu	Ser	Glu	Leu	Thr	Met	Cys	Ser	Asp	Val	Asp	Arg	Asn
		355					360				365				
Asp	Lys	Ser	Arg	Val	Cys	Glu	Pro	Gly	Ser	Val	Lys	Val	Ile	Gly	Arg
			370			375				380					
35	Arg	Gln	Ile	Glu	Met	Tyr	Ser	Arg	Leu	Ile	His	Thr	Val	Asp	His
	385					390					395				400
Glu	Gly	Arg	Leu	Arg	Asp	Asp	Met	Asp	Ala	Phe	Asp	Gly	Phe	Leu	Ser
			405						410				415		
His	Ala	Trp	Ala	Val	Thr	Val	Thr	Gly	Ala	Pro	Lys	Leu	Trp	Ala	Met
40			420					425				430			

42

```

Arg Phe Ile Glu Gly His Glu Lys Ser Pro Arg Ala Trp Tyr Gly Gly
    435                      440                      445
Ala Ile Gly Met Val Gly Phe Asn Gly Asp Met Asn Thr Gly Leu Thr
    450                      455                      460
5 Leu Arg Thr Ile Arg Ile Lys Asp Gly Ile Ala Glu Val Arg Ala Gly
  465                      470                      475                      480
Ala Thr Leu Leu Asn Asp Ser Asn Pro Gln Glu Glu Ala Glu Thr
    485                      490                      495
Glu Leu Lys Ala Ser Ala Met Ile Ser Ala Ile Arg Asp Ala Lys Gly
10                      500                      505                      510
Thr Asn Ser Ala Ala Thr Lys Arg Asp Ala Ala Lys Val Gly Thr Gly
    515                      520                      525
Val Lys Ile Leu Leu Val Asp His Glu Asp Ser Phe Val His Thr Leu
    530                      535                      540
15 Ala Asn Tyr Phe Arg Gln Thr Gly Ala Thr Val Ser Thr Val Arg Ser
  545                      550                      555                      560
Pro Val Ala Ala Asp Val Phe Asp Arg Phe Gln Pro Asp Leu Val Val
    565                      570                      575
Leu Ser Pro Gly Pro Gly Ser Pro Thr Asp Phe Asp Cys Lys Ala Thr
20                      580                      585                      590
Ile Lys Ala Ala Arg Ala Arg Asp Leu Pro Ile Phe Gly Val Cys Leu
    595                      600                      605
Gly Leu Gln Ala Leu Ala Glu Ala Tyr Gly Gly Leu Arg Gln Leu
  610                      615                      620
25 Ala Val Pro Met His Gly Lys Pro Ser Arg Ile Arg Val Leu Glu Pro
  625                      630                      635                      640
Gly Leu Val Phe Ser Gly Leu Gly Lys Glu Val Thr Val Gly Arg Tyr
    645                      650                      655
His Ser Ile Phe Ala Asp Pro Ala Thr Leu Pro Arg Asp Phe Ile Ile
30                      660                      665                      670
Thr Ala Glu Ser Glu Asp Gly Thr Ile Met Gly Ile Glu His Ala Lys
    675                      680                      685
Glu Pro Val Ala Ala Val Gln Phe His Pro Glu Ser Ile Met Thr Leu
  690                      695                      700
35 Gly Gln Asp Ala Gly Met Arg Met Ile Glu Asn Val Val Val His Leu
  705                      710                      715                      720
Thr Arg Lys Ala Lys Thr Lys Ala Ala
    725

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<211> 729

<212> PRT

<213> Artificial Sequence

5 <220>

<223> An A. tumefaciens mutant.

<400> 60

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Met Val Thr Ile Ile Gln Asp Asp Gly Ala Glu Thr Tyr Glu Thr Lys
10 1           5           10           15
Gly Gly Ile Gln Val Ser Arg Lys Arg Arg Pro Thr Asp Tyr Ala Asn
           20           25           30
Ala Ile Asp Asn Tyr Ile Glu Lys Leu Asp Ser His Arg Gly Ala Val
           35           40           45
15 Phe Ser Phe Asn Tyr Glu Tyr Pro Gly Arg Tyr Thr Arg Trp Asp Thr
           50           55           60
Ala Ile Val Asp Pro Pro Leu Gly Ile Ser Cys Phe Gly Arg Lys Met
65           70           75           80
Trp Ile Glu Ala Tyr Asn Gly Arg Gly Glu Val Leu Leu Asp Phe Ile
20           85           90           95
Thr Glu Lys Leu Lys Ala Thr Pro Asp Leu Thr Leu Gly Ala Ser Ser
           100          105          110
Thr Arg Arg Leu Asp Leu Thr Val Asn Glu Pro Asp Arg Val Phe Thr
           115          120          125
25 Glu Glu Glu Arg Ser Lys Ile Pro Thr Val Phe Thr Ala Leu Arg Ala
           130          135          140
Ile Val Asp Leu Phe Tyr Ser Ser Ala Asp Ser Ala Ile Gly Leu Phe
145          150          155          160
Gly Ala Phe Gly Tyr Asp Leu Ala Phe Gln Phe Asp Ala Ile Lys Leu
30          165          170          175
Ser Leu Ala Arg Pro Glu Asp Gln Arg Asp Met Val Leu Phe Leu Pro
           180          185          190
Asp Glu Ile Leu Val Val Asp His Tyr Ser Ala Lys Ala Trp Ile Asp
           195          200          205
35 Arg Tyr Asp Phe Glu Lys Asp Gly Met Thr Thr Asp Gly Lys Ser Ser
           210          215          220
Asp Ile Thr Pro Asp Pro Phe Lys Thr Thr Asp Thr Ile Pro Pro Lys
225          230          235          240
Gly Asp His Arg Pro Gly Glu Tyr Ser Glu Leu Val Val Lys Ala Lys
40          245          250          255

```

44

Glu Ser Phe Arg Arg Gly Asp Leu Phe Glu Val Val Pro Gly Gln Lys
 260 265 270
 Phe Met Glu Arg Cys Glu Ser Asn Pro Ser Ala Ile Ser Arg Arg Leu
 275 280 285
 5 Lys Ala Ile Asn Pro Ser Pro Tyr Ser Phe Phe Ile Asn Leu Gly Asp
 290 295 300
 Gln Glu Tyr Leu Val Gly Ala Ser Pro Glu Met Phe Val Arg Val Ser
 305 310 315 320
 Gly Arg Arg Ile Glu Thr Cys Pro Ile Ser Gly Thr Ile Lys Arg Gly
 10 325 330 335
 Asp Asp Pro Ile Ala Asp Ser Glu Gln Ile Leu Lys Leu Leu Asn Ser
 340 345 350
 Lys Lys Asp Glu Ser Glu Leu Thr Met Cys Ser Asp Val Asp Arg Asn
 355 360 365
 15 Asp Lys Ser Arg Val Cys Glu Pro Gly Ser Val Lys Val Ile Gly Arg
 370 375 380
 Arg Gln Ile Glu Met Tyr Ser Arg Leu Ile His Thr Val Asp His Ile
 385 390 395 400
 Glu Gly Arg Leu Arg Asp Asp Met Asp Ala Phe Asp Gly Phe Leu Ser
 20 405 410 415
 His Ala Trp Ala Val Thr Val Thr Gly Ala Pro Lys Leu Trp Ala Met
 420 425 430
 Arg Phe Ile Glu Gly His Glu Lys Ser Pro Arg Ala Trp Tyr Gly Gly
 435 440 445
 25 Ala Ile Gly Met Val Gly Phe Asn Gly Asp Met Asn Thr Gly Leu Thr
 450 455 460
 Leu Arg Thr Ile Arg Ile Lys Asp Gly Ile Ala Glu Val Arg Ala Gly
 465 470 475 480
 Ala Thr Leu Leu Asn Asp Ser Asn Pro Gln Glu Glu Ala Glu Thr
 30 485 490 495
 Glu Leu Lys Ala Ser Ala Met Ile Ser Ala Ile Arg Asp Ala Lys Gly
 500 505 510
 Thr Asn Ser Ala Ala Thr Lys Arg Asp Ala Ala Lys Val Gly Thr Gly
 515 520 525
 35 Val Lys Ile Leu Leu Val Asp His Glu Asp Ser Phe Val His Thr Leu
 530 535 540
 Ala Asn Tyr Phe Arg Gln Thr Gly Ala Thr Val Ser Thr Val Arg Ser
 545 550 555 560
 Pro Val Ala Ala Asp Val Phe Asp Arg Phe Gln Pro Asp Leu Val Val
 40 565 570 575

45

Leu Ser Pro Gly Pro Gly Ser Pro Thr Asp Phe Asp Cys Lys Ala Thr
 580 585 590
 Ile Lys Ala Ala Arg Ala Arg Asp Leu Pro Ile Phe Gly Val Cys Leu
 595 600 605
 5 Gly Leu Gln Ala Leu Ala Glu Ala Tyr Gly Gly Glu Leu Arg Gln Leu
 610 615 620
 Ala Val Pro Met His Gly Lys Pro Ser Arg Ile Arg Val Leu Glu Pro
 625 630 635 640
 Gly Leu Val Phe Ser Gly Leu Gly Lys Glu Val Thr Val Gly Arg Tyr
 10 645 650 655
 His Ser Ile Phe Ala Asp Pro Ala Thr Leu Pro Arg Asp Phe Ile Ile
 660 665 670
 Thr Ala Glu Ser Glu Asp Gly Thr Ile Met Gly Ile Glu His Ala Lys
 675 680 685
 15 Glu Pro Val Ala Ala Val Gln Phe His Pro Glu Ser Ile Met Thr Leu
 690 695 700
 Gly Gln Asp Ala Gly Met Arg Met Ile Glu Asn Val Val Val His Leu
 705 710 715 720
 Thr Arg Lys Ala Lys Thr Lys Ala Ala
 20 725

<210> 61

<211> 729

<212> PRT

25 <213> Artificial Sequence

<220>

<223> An A. tumefaciens mutant.

30 <400> 61

Met Val Thr Ile Ile Gln Asp Asp Gly Ala Glu Thr Tyr Glu Thr Lys
 1 5 10 15
 Gly Gly Ile Gln Val Ser Arg Lys Arg Arg Pro Thr Asp Tyr Ala Asn
 20 25 30
 35 Ala Ile Asp Asn Tyr Ile Glu Lys Leu Asp Ser His Arg Gly Ala Val
 35 40 45
 Phe Ser Cys Asn Tyr Glu Tyr Pro Gly Arg Tyr Thr Arg Trp Asp Thr
 50 55 60
 Ala Ile Val Asp Pro Pro Leu Gly Ile Ser Cys Phe Gly Arg Lys Met
 40 65 70 75 80

46

Trp Ile Glu Ala Tyr Asn Gly Arg Gly Glu Val Leu Leu Asp Phe Ile
 85 90 95
 Thr Glu Lys Leu Lys Ala Thr Pro Asp Leu Thr Leu Gly Ala Ser Ser
 100 105 110
 5 Thr Arg Arg Leu Asp Leu Thr Val Asn Glu Pro Asp Arg Val Phe Thr
 115 120 125
 Glu Glu Glu Arg Ser Lys Ile Pro Thr Val Phe Thr Ala Leu Arg Ala
 130 135 140
 Ile Val Asp Leu Phe Tyr Ser Ser Ala Asp Ser Ala Ile Gly Leu Phe
 10 145 150 155 160
 Gly Ala Phe Gly Tyr Asp Leu Ala Phe Gln Phe Asp Ala Ile Lys Leu
 165 170 175
 Ser Leu Ala Arg Pro Glu Asp Gln Arg Asp Met Val Leu Phe Leu Pro
 180 185 190
 15 Asp Glu Ile Leu Val Val Asp His Tyr Ser Ala Lys Ala Trp Ile Asp
 195 200 205
 Arg Tyr Asp Phe Glu Lys Asp Gly Met Thr Thr Asp Gly Lys Ser Ser
 210 215 220
 Asp Ile Thr Pro Asp Pro Phe Lys Thr Thr Asp Thr Ile Pro Pro Lys
 20 225 230 235 240
 Gly Asp His Arg Pro Gly Glu Tyr Ser Glu Leu Val Val Lys Ala Lys
 245 250 255
 Glu Ser Phe Arg Arg Gly Asp Leu Phe Glu Val Val Pro Gly Gln Lys
 260 265 270
 25 Phe Met Glu Arg Cys Glu Ser Asn Pro Ser Ala Ile Ser Arg Arg Leu
 275 280 285
 Lys Ala Ile Asn Pro Ser Pro Tyr Ser Phe Phe Ile Asn Leu Gly Asp
 290 295 300
 Gln Glu Tyr Leu Val Gly Ala Ser Pro Glu Met Phe Val Arg Val Ser
 30 305 310 315 320
 Gly Arg Arg Ile Glu Thr Cys Pro Ile Ser Gly Thr Ile Lys Arg Gly
 325 330 335
 Asp Asp Pro Ile Ala Asp Ser Glu Gln Ile Leu Lys Leu Leu Asn Ser
 340 345 350
 35 Lys Lys Asp Glu Ser Glu Leu Thr Met Cys Ser Asp Val Asp Arg Asn
 355 360 365
 Asp Lys Ser Arg Val Cys Glu Pro Gly Ser Val Lys Val Ile Gly Arg
 370 375 380
 Arg Gln Ile Glu Met Tyr Ser Arg Leu Ile His Thr Val Asp His Ile
 40 385 390 395 400

47

Glu Gly Arg Leu Arg Asp Asp Met Asp Ala Phe Asp Gly Phe Leu Ser
 405 410 415
 His Ala Trp Ala Val Thr Val Thr Gly Ala Pro Lys Leu Trp Ala Met
 420 425 430
 5 Arg Phe Ile Glu Gly His Glu Lys Ser Pro Arg Ala Trp Tyr Gly Gly
 435 440 445
 Ala Ile Gly Met Val Gly Phe Asn Gly Asp Met Asn Thr Gly Leu Thr
 450 455 460
 Leu Arg Thr Ile Arg Ile Lys Asp Gly Ile Ala Glu Val Arg Ala Gly
 10 465 470 475 480
 Ala Thr Leu Leu Asn Asp Ser Asn Pro Gln Glu Glu Glu Ala Glu Thr
 485 490 495
 Glu Leu Lys Ala Ser Ala Met Ile Ser Ala Ile Arg Asp Ala Lys Gly
 500 505 510
 15 Thr Asn Ser Ala Ala Thr Lys Arg Asp Ala Ala Lys Val Gly Thr Gly
 515 520 525
 Val Lys Ile Leu Leu Val Asp His Glu Asp Ser Phe Val His Thr Leu
 530 535 540
 Ala Asn Tyr Phe Arg Gln Thr Gly Ala Thr Val Ser Thr Val Arg Ser
 20 545 550 555 560
 Pro Val Ala Ala Asp Val Phe Asp Arg Phe Gln Pro Asp Leu Val Val
 565 570 575
 Leu Ser Pro Gly Pro Gly Ser Pro Thr Asp Phe Asp Cys Lys Ala Thr
 580 585 590
 25 Ile Lys Ala Ala Arg Ala Arg Asp Leu Pro Ile Phe Gly Val Cys Leu
 595 600 605
 Gly Leu Gln Ala Leu Ala Glu Ala Tyr Gly Gly Glu Leu Arg Gln Leu
 610 615 620
 Ala Val Pro Met His Gly Lys Pro Ser Arg Ile Arg Val Leu Glu Pro
 30 625 630 635 640
 Gly Leu Val Phe Ser Gly Leu Gly Lys Glu Val Thr Val Gly Arg Tyr
 645 650 655
 His Ser Ile Phe Ala Asp Pro Ala Thr Leu Pro Arg Asp Phe Ile Ile
 660 665 670
 35 Thr Ala Glu Ser Glu Asp Gly Thr Ile Met Gly Ile Glu His Ala Lys
 675 680 685
 Glu Pro Val Ala Ala Val Gln Phe His Pro Glu Ser Ile Met Thr Leu
 690 695 700
 Gly Gln Asp Ala Gly Met Arg Met Ile Glu Asn Val Val Val His Leu
 40 705 710 715 720

48

Thr Arg Lys Ala Lys Thr Lys Ala Ala
725

<210> 62

5 <211> 729

<212> PRT

<213> Artificial Sequence

<220>

10 <223> An A. tumefaciens mutant.

<400> 62

Met	Val	Thr	Ile	Ile	Gln	Asp	Asp	Gly	Ala	Glu	Thr	Tyr	Glu	Thr	Lys
1				5					10					15	
15	Gly	Gly	Ile	Gln	Val	Ser	Arg	Lys	Arg	Arg	Pro	Thr	Asp	Tyr	Ala
			20						25					30	Asn
	Ala	Ile	Asp	Asn	Tyr	Ile	Glu	Lys	Leu	Asp	Ser	His	Arg	Gly	Ala
			35						40				45		Val
	Phe	Ser	Ser	Phe	Tyr	Glu	Tyr	Pro	Gly	Arg	Tyr	Thr	Arg	Trp	Asp
20		50					55					60			Thr
	Ala	Ile	Val	Asp	Pro	Pro	Leu	Gly	Ile	Ser	Cys	Phe	Gly	Arg	Lys
65					70					75				80	Met
	Trp	Ile	Glu	Ala	Tyr	Asn	Gly	Arg	Gly	Glu	Val	Leu	Leu	Asp	Phe
					85					90				95	Ile
25	Thr	Glu	Lys	Leu	Lys	Ala	Thr	Pro	Asp	Leu	Thr	Leu	Gly	Ala	Ser
					100					105				110	Ser
	Thr	Arg	Arg	Leu	Asp	Leu	Thr	Val	Asn	Glu	Pro	Asp	Arg	Val	Phe
					115					120				125	Thr
	Glu	Glu	Glu	Arg	Ser	Lys	Ile	Pro	Thr	Val	Phe	Thr	Ala	Leu	Arg
30		130					135						140		Ala
	Ile	Val	Asp	Leu	Phe	Tyr	Ser	Ser	Ala	Asp	Ser	Ala	Ile	Gly	Leu
145						150					155			160	Phe
	Gly	Ala	Phe	Gly	Tyr	Asp	Leu	Ala	Phe	Gln	Phe	Asp	Ala	Ile	Lys
					165					170				175	Leu
35	Ser	Leu	Ala	Arg	Pro	Glu	Asp	Gln	Arg	Asp	Met	Val	Leu	Phe	Leu
					180					185				190	Pro
	Asp	Glu	Ile	Leu	Val	Val	Asp	His	Tyr	Ser	Ala	Lys	Ala	Trp	Ile
					195					200				205	Asp
	Arg	Tyr	Asp	Phe	Glu	Lys	Asp	Gly	Met	Thr	Thr	Asp	Gly	Lys	Ser
40		210								215				220	Ser

49

	Asp	Ile	Thr	Pro	Asp	Pro	Phe	Lys	Thr	Thr	Asp	Thr	Ile	Pro	Pro	Lys
225						230					235					240
	Gly	Asp	His	Arg	Pro	Gly	Glu	Tyr	Ser	Glu	Leu	Val	Val	Lys	Ala	Lys
					245					250					255	
5	Glu	Ser	Phe	Arg	Arg	Gly	Asp	Leu	Phe	Glu	Val	Val	Pro	Gly	Gln	Lys
					260					265					270	
	Phe	Met	Glu	Arg	Cys	Glu	Ser	Asn	Pro	Ser	Ala	Ile	Ser	Arg	Arg	Leu
					275				280				285			
	Lys	Ala	Ile	Asn	Pro	Ser	Pro	Tyr	Ser	Phe	Phe	Ile	Asn	Leu	Gly	Asp
10		290						295				300				
	Gln	Glu	Tyr	Leu	Val	Gly	Ala	Ser	Pro	Glu	Met	Phe	Val	Arg	Val	Ser
					305						315					320
	Gly	Arg	Arg	Ile	Glu	Thr	Cys	Pro	Ile	Ser	Gly	Thr	Ile	Lys	Arg	Gly
					325					330					335	
15	Asp	Asp	Pro	Ile	Ala	Asp	Ser	Glu	Gln	Ile	Leu	Lys	Leu	Leu	Asn	Ser
					340					345					350	
	Lys	Lys	Asp	Glu	Ser	Glu	Leu	Thr	Met	Cys	Ser	Asp	Val	Asp	Arg	Asn
					355				360				365			
	Asp	Lys	Ser	Arg	Val	Cys	Glu	Pro	Gly	Ser	Val	Lys	Val	Ile	Gly	Arg
20		370						375				380				
	Arg	Gln	Ile	Glu	Met	Tyr	Ser	Arg	Leu	Ile	His	Thr	Val	Asp	His	Ile
					385						395					400
	Glu	Gly	Arg	Leu	Arg	Asp	Asp	Met	Asp	Ala	Phe	Asp	Gly	Phe	Leu	Ser
					405					410					415	
25	His	Ala	Trp	Ala	Val	Thr	Val	Thr	Gly	Ala	Pro	Lys	Leu	Trp	Ala	Met
					420					425					430	
	Arg	Phe	Ile	Glu	Gly	His	Glu	Lys	Ser	Pro	Arg	Ala	Trp	Tyr	Gly	Gly
					435				440				445			
	Ala	Ile	Gly	Met	Val	Gly	Phe	Asn	Gly	Asp	Met	Asn	Thr	Gly	Leu	Thr
30		450						455				460				
	Leu	Arg	Thr	Ile	Arg	Ile	Lys	Asp	Gly	Ile	Ala	Glu	Val	Arg	Ala	Gly
					465						475					480
	Ala	Thr	Leu	Leu	Asn	Asp	Ser	Asn	Pro	Gln	Glu	Glu	Glu	Ala	Glu	Thr
					485					490					495	
35	Glu	Leu	Lys	Ala	Ser	Ala	Met	Ile	Ser	Ala	Ile	Arg	Asp	Ala	Lys	Gly
					500					505					510	
	Thr	Asn	Ser	Ala	Ala	Thr	Lys	Arg	Asp	Ala	Ala	Lys	Val	Gly	Thr	Gly
					515				520				525			
	Val	Lys	Ile	Leu	Leu	Val	Asp	His	Glu	Asp	Ser	Phe	Val	His	Thr	Leu
40		530						535				540				

50

Ala Asn Tyr Phe Arg Gln Thr Gly Ala Thr Val Ser Thr Val Arg Ser
 545 550 555 560
 Pro Val Ala Ala Asp Val Phe Asp Arg Phe Gln Pro Asp Leu Val Val
 565 570 575
 5 Leu Ser Pro Gly Pro Gly Ser Pro Thr Asp Phe Asp Cys Lys Ala Thr
 580 585 590
 Ile Lys Ala Ala Arg Ala Arg Asp Leu Pro Ile Phe Gly Val Cys Leu
 595 600 605
 Gly Leu Gln Ala Leu Ala Glu Ala Tyr Gly Gly Glu Leu Arg Gln Leu
 10 610 615 620
 Ala Val Pro Met His Gly Lys Pro Ser Arg Ile Arg Val Leu Glu Pro
 625 630 635 640
 Gly Leu Val Phe Ser Gly Leu Gly Lys Glu Val Thr Val Gly Arg Tyr
 645 650 655
 15 His Ser Ile Phe Ala Asp Pro Ala Thr Leu Pro Arg Asp Phe Ile Ile
 660 665 670
 Thr Ala Glu Ser Glu Asp Gly Thr Ile Met Gly Ile Glu His Ala Lys
 675 680 685
 Glu Pro Val Ala Ala Val Gln Phe His Pro Glu Ser Ile Met Thr Leu
 20 690 695 700
 Gly Gln Asp Ala Gly Met Arg Met Ile Glu Asn Val Val Val His Leu
 705 710 715 720
 Thr Arg Lys Ala Lys Thr Lys Ala Ala
 725

25

<210> 63
 <211> 729
 <212> PRT
 <213> Artificial Sequence

30

<220>
 <223> An A. tumefaciens mutant.

<400> 63

35 Met Val Thr Ile Ile Gln Asp Asp Gly Ala Glu Thr Tyr Glu Thr Lys
 1 5 10 15
 Gly Gly Ile Gln Val Ser Arg Lys Arg Arg Pro Thr Asp Tyr Ala Asn
 20 25 30
 Ala Ile Asp Asn Tyr Ile Glu Lys Leu Asp Ser His Arg Gly Ala Val
 40 35 40 45

51

Phe	Ser	Ser	Asn	Tyr	Glu	Tyr	Pro	Gly	Arg	Tyr	Thr	Arg	Trp	Asp	Thr
50						55					60				
Ala	Ile	Val	Asp	Pro	Pro	Leu	Gly	Ile	Ser	Cys	Phe	Gly	Arg	Lys	Met
65					70					75				80	
5	Trp	Ile	Glu	Ala	Tyr	Asn	Gly	Arg	Gly	Glu	Val	Leu	Leu	Asp	Phe
					85					90				95	
Thr	Glu	Lys	Leu	Lys	Ala	Thr	Pro	Asp	Leu	Thr	Leu	Gly	Ala	Ser	Ser
			100						105				110		
Thr	Arg	Arg	Leu	Asp	Leu	Thr	Val	Asn	Glu	Pro	Asp	Arg	Val	Phe	Thr
10			115					120				125			
Glu	Glu	Glu	Arg	Ser	Lys	Ile	Pro	Thr	Val	Phe	Thr	Ala	Leu	Arg	Ala
			130					135				140			
Ile	Val	Asp	Leu	Phe	Tyr	Ser	Ser	Ala	Asp	Ser	Ala	Ile	Gly	Leu	Phe
145					150					155				160	
15	Gly	Ala	Phe	Gly	Tyr	Asp	Leu	Ala	Phe	Gln	Phe	Asp	Ala	Ile	Lys
					165					170				175	
Ser	Leu	Ala	Arg	Pro	Glu	Asp	Gln	Arg	Asp	Met	Val	Leu	Phe	Leu	Pro
			180					185				190			
Asp	Glu	Ile	Leu	Val	Val	Asp	His	Tyr	Ser	Ala	Lys	Ala	Trp	Ile	Asp
20			195					200				205			
Arg	Tyr	Asp	Phe	Glu	Lys	Asp	Gly	Met	Thr	Thr	Asp	Gly	Lys	Ser	Ser
			210					215				220			
Asp	Ile	Thr	Pro	Asp	Pro	Phe	Lys	Thr	Thr	Asp	Thr	Ile	Pro	Pro	Lys
225					230					235				240	
25	Gly	Asp	His	Arg	Pro	Gly	Glu	Tyr	Ser	Glu	Leu	Val	Val	Lys	Ala
					245					250				255	
Glu	Ser	Phe	Arg	Arg	Gly	Asp	Leu	Phe	Glu	Val	Val	Pro	Gly	Gln	Lys
			260					265				270			
Phe	Met	Glu	Arg	Cys	Glu	Ser	Asn	Pro	Ser	Ala	Ile	Ser	Arg	Arg	Leu
30			275					280				285			
Lys	Ala	Ile	Asn	Ala	Ser	Pro	Tyr	Ser	Phe	Phe	Ile	Asn	Leu	Gly	Asp
			290					295				300			
Gln	Glu	Tyr	Leu	Val	Gly	Ala	Ser	Pro	Glu	Met	Phe	Val	Arg	Val	Ser
305					310					315				320	
35	Gly	Arg	Arg	Ile	Glu	Thr	Cys	Pro	Ile	Ser	Gly	Thr	Ile	Lys	Arg
					325					330				335	
Asp	Asp	Pro	Ile	Ala	Asp	Ser	Glu	Gln	Ile	Leu	Lys	Leu	Leu	Asn	Ser
			340					345				350			
Lys	Lys	Asp	Glu	Ser	Glu	Leu	Thr	Met	Cys	Ser	Asp	Val	Asp	Arg	Asn
40			355					360				365			

52

Asp Lys Ser Arg Val Cys Glu Pro Gly Ser Val Lys Val Ile Gly Arg
 370 375 380
 Arg Gln Ile Glu Met Tyr Ser Arg Leu Ile His Thr Val Asp His Ile
 385 390 395 400
 5 Glu Gly Arg Leu Arg Asp Asp Met Asp Ala Phe Asp Gly Phe Leu Ser
 405 410 415
 His Ala Trp Ala Val Thr Val Thr Gly Ala Pro Lys Leu Trp Ala Met
 420 425 430
 Arg Phe Ile Glu Gly His Glu Lys Ser Pro Arg Ala Trp Tyr Gly Gly
 10 435 440 445
 Ala Ile Gly Met Val Gly Phe Asn Gly Asp Met Asn Thr Gly Leu Thr
 450 455 460
 Leu Arg Thr Ile Arg Ile Lys Asp Gly Ile Ala Glu Val Arg Ala Gly
 465 470 475 480
 15 Ala Thr Leu Leu Asn Asp Ser Asn Pro Gln Glu Glu Glu Ala Glu Thr
 485 490 495
 Glu Leu Lys Ala Ser Ala Met Ile Ser Ala Ile Arg Asp Ala Lys Gly
 500 505 510
 Thr Asn Ser Ala Ala Thr Lys Arg Asp Ala Ala Lys Val Gly Thr Gly
 20 515 520 525
 Val Lys Ile Leu Leu Val Asp His Glu Asp Ser Phe Val His Thr Leu
 530 535 540
 Ala Asn Tyr Phe Arg Gln Thr Gly Ala Thr Val Ser Thr Val Arg Ser
 545 550 555 560
 25 Pro Val Ala Ala Asp Val Phe Asp Arg Phe Gln Pro Asp Leu Val Val
 565 570 575
 Leu Ser Pro Gly Pro Gly Ser Pro Thr Asp Phe Asp Cys Lys Ala Thr
 580 585 590
 Ile Lys Ala Ala Arg Ala Arg Asp Leu Pro Ile Phe Gly Val Cys Leu
 30 595 600 605
 Gly Leu Gln Ala Leu Ala Glu Ala Tyr Gly Gly Glu Leu Arg Gln Leu
 610 615 620
 Ala Val Pro Met His Gly Lys Pro Ser Arg Ile Arg Val Leu Glu Pro
 625 630 635 640
 35 Gly Leu Val Phe Ser Gly Leu Gly Lys Glu Val Thr Val Gly Arg Tyr
 645 650 655
 His Ser Ile Phe Ala Asp Pro Ala Thr Leu Pro Arg Asp Phe Ile Ile
 660 665 670
 Thr Ala Glu Ser Glu Asp Gly Thr Ile Met Gly Ile Glu His Ala Lys
 40 675 680 685

53

Glu Pro Val Ala Ala Val Gln Phe His Pro Glu Ser Ile Met Thr Leu
 690 695 700
 Gly Gln Asp Ala Gly Met Arg Met Ile Glu Asn Val Val Val His Leu
 705 710 715 720
 5 Thr Arg Lys Ala Lys Thr Lys Ala Ala
 725

<210> 64

<211> 729

10 <212> PRT

<213> Artificial Sequence

<220>

<223> An A. tumefaciens mutant.

15

<400> 64

Met Val Thr Ile Ile Gln Asp Asp Gly Ala Glu Thr Tyr Glu Thr Lys
 1 5 10 15
 Gly Gly Ile Gln Val Ser Arg Lys Arg Arg Pro Thr Asp Tyr Ala Asn
 20 20 25 30
 Ala Ile Asp Asn Tyr Ile Glu Lys Leu Asp Ser His Arg Gly Ala Val
 35 40 45
 Phe Ser Ser Asn Tyr Glu Tyr Pro Gly Arg Tyr Thr Arg Trp Asp Thr
 50 55 60
 25 Ala Ile Val Asp Pro Pro Leu Gly Ile Ser Cys Phe Gly Arg Lys Met
 65 70 75 80
 Trp Ile Glu Ala Tyr Asn Gly Arg Gly Glu Val Leu Leu Asp Phe Ile
 85 90 95
 Thr Glu Lys Leu Lys Ala Thr Pro Asp Leu Thr Leu Gly Ala Ser Ser
 30 100 105 110
 Thr Arg Arg Leu Asp Leu Thr Val Asn Glu Pro Asp Arg Val Phe Thr
 115 120 125
 Glu Glu Glu Arg Ser Lys Ile Pro Thr Val Phe Thr Ala Leu Arg Ala
 130 135 140
 35 Ile Val Asp Leu Phe Tyr Ser Ser Ala Asp Ser Ala Ile Gly Leu Phe
 145 150 155 160
 Gly Ala Phe Gly Tyr Asp Leu Ala Phe Gln Phe Asp Ala Ile Lys Leu
 165 170 175
 Ser Leu Ala Arg Pro Glu Asp Gln Arg Asp Met Val Leu Phe Leu Pro
 40 180 185 190

54

Asp Glu Ile Leu Val Val Asp His Tyr Ser Ala Lys Ala Trp Ile Asp
 195 200 205
 Arg Tyr Asp Phe Glu Lys Asp Gly Met Thr Thr Asp Gly Lys Ser Ser
 210 215 220
 5 Asp Ile Thr Pro Asp Pro Phe Lys Thr Thr Asp Thr Ile Pro Pro Lys
 225 230 235 240
 Gly Asp His Arg Pro Gly Glu Tyr Ser Glu Leu Val Val Lys Ala Lys
 245 250 255
 Glu Ser Phe Arg Arg Gly Asp Leu Phe Glu Val Val Pro Gly Gln Lys
 10 260 265 270
 Phe Met Glu Arg Cys Glu Ser Asn Pro Ser Ala Ile Ser Arg Arg Leu
 275 280 285
 Lys Ala Ile Asn Gly Ser Pro Tyr Ser Phe Phe Ile Asn Leu Gly Asp
 290 295 300
 15 Gln Glu Tyr Leu Val Gly Ala Ser Pro Glu Met Phe Val Arg Val Ser
 305 310 315 320
 Gly Arg Arg Ile Glu Thr Cys Pro Ile Ser Gly Thr Ile Lys Arg Gly
 325 330 335
 Asp Asp Pro Ile Ala Asp Ser Glu Gln Ile Leu Lys Leu Leu Asn Ser
 20 340 345 350
 Lys Lys Asp Glu Ser Glu Leu Thr Met Cys Ser Asp Val Asp Arg Asn
 355 360 365
 Asp Lys Ser Arg Val Cys Glu Pro Gly Ser Val Lys Val Ile Gly Arg
 370 375 380
 25 Arg Gln Ile Glu Met Tyr Ser Arg Leu Ile His Thr Val Asp His Ile
 385 390 395 400
 Glu Gly Arg Leu Arg Asp Asp Met Asp Ala Phe Asp Gly Phe Leu Ser
 405 410 415
 His Ala Trp Ala Val Thr Val Thr Gly Ala Pro Lys Leu Trp Ala Met
 30 420 425 430
 Arg Phe Ile Glu Gly His Glu Lys Ser Pro Arg Ala Trp Tyr Gly Gly
 435 440 445
 Ala Ile Gly Met Val Gly Phe Asn Gly Asp Met Asn Thr Gly Leu Thr
 450 455 460
 35 Leu Arg Thr Ile Arg Ile Lys Asp Gly Ile Ala Glu Val Arg Ala Gly
 465 470 475 480
 Ala Thr Leu Leu Asn Asp Ser Asn Pro Gln Glu Glu Glu Ala Glu Thr
 485 490 495
 Glu Leu Lys Ala Ser Ala Met Ile Ser Ala Ile Arg Asp Ala Lys Gly
 40 500 505 510

55

Thr Asn Ser Ala Ala Thr Lys Arg Asp Ala Ala Lys Val Gly Thr Gly
 515 520 525
 Val Lys Ile Leu Leu Val Asp His Glu Asp Ser Phe Val His Thr Leu
 530 535 540
 5 Ala Asn Tyr Phe Arg Gln Thr Gly Ala Thr Val Ser Thr Val Arg Ser
 545 550 555 560
 Pro Val Ala Ala Asp Val Phe Asp Arg Phe Gln Pro Asp Leu Val Val
 565 570 575
 Leu Ser Pro Gly Pro Gly Ser Pro Thr Asp Phe Asp Cys Lys Ala Thr
 10 580 585 590
 Ile Lys Ala Ala Arg Ala Arg Asp Leu Pro Ile Phe Gly Val Cys Leu
 595 600 605
 Gly Leu Gln Ala Leu Ala Glu Ala Tyr Gly Gly Glu Leu Arg Gln Leu
 610 615 620
 15 Ala Val Pro Met His Gly Lys Pro Ser Arg Ile Arg Val Leu Glu Pro
 625 630 635 640
 Gly Leu Val Phe Ser Gly Leu Gly Lys Glu Val Thr Val Gly Arg Tyr
 645 650 655
 His Ser Ile Phe Ala Asp Pro Ala Thr Leu Pro Arg Asp Phe Ile Ile
 20 660 665 670
 Thr Ala Glu Ser Glu Asp Gly Thr Ile Met Gly Ile Glu His Ala Lys
 675 680 685
 Glu Pro Val Ala Ala Val Gln Phe His Pro Glu Ser Ile Met Thr Leu
 690 695 700
 25 Gly Gln Asp Ala Gly Met Arg Met Ile Glu Asn Val Val Val His Leu
 705 710 715 720
 Thr Arg Lys Ala Lys Thr Lys Ala Ala
 725

30 <210> 65

<211> 729

<212> PRT

<213> Artificial Sequence

35 <220>

<223> An A. tumefaciens mutant.

<400> 65

Met Val Thr Ile Ile Gln Asp Asp Gly Ala Glu Thr Tyr Glu Thr Lys

40 1

5

10

15

56

Gly Gly Ile Gln Val Ser Arg Lys Arg Arg Pro Thr Asp Tyr Ala Asn
 20 25 30
 Ala Ile Asp Asn Tyr Ile Glu Lys Leu Asp Ser His Arg Gly Ala Val
 35 40 45
 5 Phe Ser Ser Asn Tyr Glu Tyr Pro Gly Arg Tyr Thr Arg Trp Asp Thr
 50 55 60
 Ala Ile Val Asp Pro Pro Leu Gly Ile Ser Cys Phe Gly Arg Lys Met
 65 70 75 80
 Trp Ile Glu Ala Tyr Asn Gly Arg Gly Glu Val Leu Leu Asp Phe Ile
 10 85 90 95
 Thr Glu Lys Leu Lys Ala Thr Pro Asp Leu Thr Leu Gly Ala Ser Ser
 100 105 110
 Thr Arg Arg Leu Asp Leu Thr Val Asn Glu Pro Asp Arg Val Phe Thr
 115 120 125
 15 Glu Glu Glu Arg Ser Lys Ile Pro Thr Val Phe Thr Ala Leu Arg Ala
 130 135 140
 Ile Val Asp Leu Phe Tyr Ser Ser Ala Asp Ser Ala Ile Gly Leu Phe
 145 150 155 160
 Gly Ala Phe Gly Tyr Asp Leu Ala Phe Gln Phe Asp Ala Ile Lys Leu
 20 165 170 175
 Ser Leu Ala Arg Pro Glu Asp Gln Arg Asp Met Val Leu Phe Leu Pro
 180 185 190
 Asp Glu Ile Leu Val Val Asp His Tyr Ser Ala Lys Ala Trp Ile Asp
 195 200 205
 25 Arg Tyr Asp Phe Glu Lys Asp Gly Met Thr Thr Asp Gly Lys Ser Ser
 210 215 220
 Asp Ile Thr Pro Asp Pro Phe Lys Thr Thr Asp Thr Ile Pro Pro Lys
 225 230 235 240
 Gly Asp His Arg Pro Gly Glu Tyr Ser Glu Leu Val Val Lys Ala Lys
 30 245 250 255
 Glu Ser Phe Arg Arg Gly Asp Leu Phe Glu Val Val Pro Gly Gln Lys
 260 265 270
 Phe Met Glu Arg Cys Glu Ser Asn Pro Ser Ala Ile Ser Arg Arg Leu
 275 280 285
 35 Lys Ala Ile Asn Pro Ser Pro Tyr Ser Trp Phe Ile Asn Leu Gly Asp
 290 295 300
 Gln Glu Tyr Leu Val Gly Ala Ser Pro Glu Met Phe Val Arg Val Ser
 305 310 315 320
 Gly Arg Arg Ile Glu Thr Cys Pro Ile Ser Gly Thr Ile Lys Arg Gly
 40 325 330 335

57

Asp Asp Pro Ile Ala Asp Ser Glu Gln Ile Leu Lys Leu Leu Asn Ser
 340 345 350
 Lys Lys Asp Glu Ser Glu Leu Thr Met Cys Ser Asp Val Asp Arg Asn
 355 360 365
 5 Asp Lys Ser Arg Val Cys Glu Pro Gly Ser Val Lys Val Ile Gly Arg
 370 375 380
 Arg Gln Ile Glu Met Tyr Ser Arg Leu Ile His Thr Val Asp His Ile
 385 390 395 400
 Glu Gly Arg Leu Arg Asp Asp Met Asp Ala Phe Asp Gly Phe Leu Ser
 10 405 410 415
 His Ala Trp Ala Val Thr Val Thr Gly Ala Pro Lys Leu Trp Ala Met
 420 425 430
 Arg Phe Ile Glu Gly His Glu Lys Ser Pro Arg Ala Trp Tyr Gly Gly
 435 440 445
 15 Ala Ile Gly Met Val Gly Phe Asn Gly Asp Met Asn Thr Gly Leu Thr
 450 455 460
 Leu Arg Thr Ile Arg Ile Lys Asp Gly Ile Ala Glu Val Arg Ala Gly
 465 470 475 480
 Ala Thr Leu Leu Asn Asp Ser Asn Pro Gln Glu Glu Glu Ala Glu Thr
 20 485 490 495
 Glu Leu Lys Ala Ser Ala Met Ile Ser Ala Ile Arg Asp Ala Lys Gly
 500 505 510
 Thr Asn Ser Ala Ala Thr Lys Arg Asp Ala Ala Lys Val Gly Thr Gly
 515 520 525
 25 Val Lys Ile Leu Leu Val Asp His Glu Asp Ser Phe Val His Thr Leu
 530 535 540
 Ala Asn Tyr Phe Arg Gln Thr Gly Ala Thr Val Ser Thr Val Arg Ser
 545 550 555 560
 Pro Val Ala Ala Asp Val Phe Asp Arg Phe Gln Pro Asp Leu Val Val
 30 565 570 575
 Leu Ser Pro Gly Pro Gly Ser Pro Thr Asp Phe Asp Cys Lys Ala Thr
 580 585 590
 Ile Lys Ala Ala Arg Ala Arg Asp Leu Pro Ile Phe Gly Val Cys Leu
 595 600 605
 35 Gly Leu Gln Ala Leu Ala Glu Ala Tyr Gly Gly Glu Leu Arg Gln Leu
 610 615 620
 Ala Val Pro Met His Gly Lys Pro Ser Arg Ile Arg Val Leu Glu Pro
 625 630 635 640
 Gly Leu Val Phe Ser Gly Leu Gly Lys Glu Val Thr Val Gly Arg Tyr
 40 645 650 655

58

His Ser Ile Phe Ala Asp Pro Ala Thr Leu Pro Arg Asp Phe Ile Ile
 660 665 670
 Thr Ala Glu Ser Glu Asp Gly Thr Ile Met Gly Ile Glu His Ala Lys
 675 680 685
 5 Glu Pro Val Ala Ala Val Gln Phe His Pro Glu Ser Ile Met Thr Leu
 690 695 700
 Gly Gln Asp Ala Gly Met Arg Met Ile Glu Asn Val Val Val His Leu
 705 710 715 720
 Thr Arg Lys Ala Lys Thr Lys Ala Ala
 10 725

<210> 66

<211> 604

<212> PRT

15 <213> Artificial Sequence

<220>

<223> A Zea mays mutant.

20 <400> 66

Met Glu Ser Leu Ala Ala Thr Ser Val Phe Ala Pro Ser Arg Val Ala
 1 5 10 15
 Val Pro Ala Ala Arg Ala Leu Val Arg Ala Gly Thr Val Val Pro Thr
 20 25 30
 25 Arg Arg Thr Ser Ser Arg Ser Gly Thr Ser Gly Val Lys Cys Ser Ala
 35 40 45
 Ala Val Thr Pro Gln Ala Ser Pro Val Ile Ser Arg Ser Ala Ala Ala
 50 55 60
 Ala Lys Ala Ala Glu Glu Asp Lys Arg Arg Phe Phe Glu Ala Ala Ala
 30 65 70 75 80
 Arg Gly Ser Gly Lys Gly Asn Leu Val Pro Met Trp Glu Cys Ile Val
 85 90 95
 Ser Asp His Leu Thr Pro Val Leu Ala Tyr Arg Cys Leu Val Pro Glu
 100 105 110
 35 Asp Asn Val Asp Ala Pro Ser Phe Leu Phe Glu Ser Val Glu Gln Gly
 115 120 125
 Pro Gln Gly Thr Thr Asn Val Gly Arg Tyr Ser Met Val Gly Ala His
 130 135 140
 Pro Val Met Glu Ile Val Ala Lys Asp His Lys Val Thr Ile Met Asp
 40 145 150 155 160

59

His Glu Lys Ser Gln Val Thr Glu Gln Val Val Asp Asp Pro Met Gln
 165 170 175
 Ile Pro Arg Thr Met Met Glu Gly Trp His Pro Gln Gln Ile Asp Glu
 180 185 190
 5 Leu Pro Glu Ser Phe Ser Gly Gly Trp Val Gly Phe Phe Ser Tyr Asp
 195 200 205
 Thr Val Arg Tyr Val Glu Lys Lys Lys Leu Pro Phe Ser Ser Ala Pro
 210 215 220
 Gln Asp Asp Arg Asn Leu Pro Asp Val His Leu Gly Leu Tyr Asp Asp
 10 225 230 235 240
 Val Leu Val Phe Asp Asn Val Glu Lys Lys Val Tyr Val Ile His Trp
 245 250 255
 Val Asn Val Asp Arg His Ala Ser Val Glu Glu Ala Tyr Gln Asp Gly
 260 265 270
 15 Arg Ser Arg Leu Asn Met Leu Leu Ser Lys Val His Asn Ser Asn Val
 275 280 285
 Pro Thr Leu Ser Pro Gly Phe Val Lys Leu His Thr Arg Lys Phe Gly
 290 295 300
 Thr Pro Leu Asn Lys Ser Thr Met Thr Ser Asp Glu Tyr Lys Asn Ala
 20 305 310 315 320
 Val Leu Gln Ala Lys Glu His Ile Met Ala Gly Asp Ile Phe Gln Ile
 325 330 335
 Val Leu Ser Gln Arg Phe Glu Arg Arg Thr Tyr Ala Asn Pro Phe Glu
 340 345 350
 25 Val Tyr Arg Ala Leu Arg Ile Val Asn Pro Ser Pro Tyr Lys Ala Tyr
 355 360 365
 Val Gln Ala Arg Gly Cys Val Leu Val Ala Ser Ser Pro Glu Ile Leu
 370 375 380
 Thr Arg Val Ser Lys Gly Lys Ile Ile Asn Arg Pro Leu Ala Gly Thr
 30 385 390 395 400
 Val Arg Arg Gly Lys Thr Glu Lys Glu Asp Gln Met Gln Glu Gln Gln
 405 410 415
 Leu Leu Ser Asp Glu Lys Gln Cys Ala Glu His Ile Met Leu Val Asp
 420 425 430
 35 Leu Gly Arg Asn Asp Val Gly Lys Val Ser Lys Pro Gly Ser Val Lys
 435 440 445
 Val Glu Lys Leu Met Asn Ile Glu Arg Tyr Ser His Val Met His Ile
 450 455 460
 Ser Ser Thr Val Ser Gly Gln Leu Asp Asp His Leu Gln Ser Trp Asp
 40 465 470 475 480

60

Ala Leu Arg Ala Ala Leu Pro Val Gly Thr Val Ser Gly Ala Pro Lys
 485 490 495
 Val Lys Ala Met Glu Leu Ile Asp Lys Leu Glu Val Thr Arg Arg Gly
 500 505 510
 5 Pro Tyr Ser Gly Gly Leu Gly Gly Ile Ser Phe Asp Gly Asp Met Gln
 515 520 525
 Ile Ala Leu Ser Leu Arg Thr Ile Val Phe Ser Thr Ala Pro Ser His
 530 535 540
 Asn Thr Met Tyr Ser Tyr Lys Asp Ala Asp Arg Arg Arg Glu Trp Val
 10 545 550 555 560
 Ala His Leu Gln Ala Gly Ala Gly Ile Val Ala Asp Ser Ser Pro Asp
 565 570 575
 Asp Glu Gln Arg Glu Cys Glu Asn Lys Ala Ala Ala Leu Ala Arg Ala
 580 585 590
 15 Ile Asp Leu Ala Glu Ser Ala Phe Val Asp Lys Glu
 595 600

<210> 67

<211> 1815

20 <212> DNA

<213> Artificial Sequence

<220>

<223> A Zea mays mutant.

25

<400> 67

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 accagcgggg tgaatgctc tgctgccgtg acgcgcaggc cgagccagct gattagcagg 180
 30 agcgcctcgg cggcgaaggc ggcggaggag gacaagaggc ggtctctoga ggcgcggcgg 240
 cgggggagcg ggaaggggaa cctgggtccc atgtgggagt gcctcgtgtc ggaccatctc 300
 acccccgctgc tcgcctaccg ctgcctcgtc cccgaggaca acgtcgacgc cccacgcttc 360
 ctcttcgagt ccgtcgagca ggggcccccag ggcaccacca acgtcggcgg ctatagcatg 420
 gtgggagccc acccagtgat ggagattgtg gccaaagacc acaagggttac gatcatggac 480
 35 caccagaaga gccaaagtgc agagcaggta gtggacgacc cgatgcagat cccgaggacc 540
 atgatggagg gatggcacc acagcagatc gacgagctcc ctgaatcott ctccggtgga 600
 tgggttggtt tcttttctta tgatagcgtt aggtatgttg agaagaagaa gctaccgttc 660
 tccagtgtct ctcaggacga taggaacctt cctgatgtgc acttgggact ctatgatgat 720
 gttctagtct tcgataatgt tgagaagaaa gtatatgtta tccattgggt caatgtggac 780
 40 cggcatgcat ctggttgagg agcataccaa gatggcaggc cccgactaaa catgttgcta 840

tctaagatgc	acaattccaa	tgccccaca	ctctctctcg	gatttgtgaa	gctgcacaca	900
cgaagtttg	gtacaccttt	gaacaagtcg	accatgacaa	gtgatgagta	taagaatgct	960
gtttctgcagg	ctaaggaaca	tattatggct	gggatatatct	tccagattgt	tttaagccag	1020
aggttcgaga	gacgaacata	tgccaaccca	tttgagggtt	atcgagcatt	acggattgtg	1080
5 aatcctagcc	catacaaggc	gtatgtacag	gcaagaggct	gtgtattggt	tgctctctagt	1140
cctgaaatc	ttacacgagt	cagtaagggg	aagattatta	atcgaccact	tgctggaact	1200
gttcgaaggg	gcaagacaga	gaaggaagat	caaatgcaag	agcagcaact	gttaagtgtat	1260
gaaaaacagt	gtgccgagca	cataatgctt	gtggaacttg	gaaggaatga	tgttgccaag	1320
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10 gtatgcaca	tcagctcaac	ggttagtggg	cagttggatg	atcatctcca	gagttgggat	1440
gccttgagag	ctgccttgcc	cgttggaaca	gtcagtggtg	caccaaagg	gaaggccatg	1500
gagttgattg	ataagttgga	agttacgagg	cgaggacccat	atagtggtgg	tctaggagga	1560
atctcgtttg	atggtgacat	gcaaatgca	ctttctctcc	gcaccatcgt	attctcaaca	1620
gcgcgagacc	acaacacgat	gtactcatac	aaagacgcag	ataggcgtcg	ggagtgggtc	1680
15 gctcatcttc	aggtcgtgtc	aggcattgtt	gcgcacagta	gccagatga	cgaacaacgt	1740
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<210> 68

20 <211> 2204

<212> DNA

<213> Artificial Sequence

<220>

25 <223> A Zea mays mutant.

<400> 68

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30 accagcgggg	tgaatgtctc	tgctgccgtg	acgccgcagg	cgagcccagt	gattagcagg	180
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cgggggagcg	ggaaggggaa	cctggtgcc	atgtggggagt	gcactgtgtc	ggaccatctc	300
acccccgtgc	tcgcctaccg	ctgcctcgtc	cccaggagaa	acgtcgagc	ccccagcttc	360
ctcttcgagt	ccgtcgagca	ggggcccccag	ggcaccacca	acgtcgcccg	ctatagcatg	420
35 ttggggagccc	accagtgat	ggagattgtg	gccaagacc	acaagggttac	gatcatggac	480
cacgagaaga	gccaaagtgc	agagcaggta	gtggacgacc	cgatgcagat	cccaggagacc	540
atgatggagg	gatggcacc	acagcagatc	gacgagctcc	ctgaatcctt	ctccggtgga	600
ttgggtgggt	tcttttctta	tgatacgggt	aggtatgttg	agaagaagaa	gctaccgttc	660
tcagtgctc	ctcaggagca	taggaacctt	cctgatgtgc	acttgggact	ctatgatgat	720
40 gttctagtct	tcgataatgt	tgagaagaaa	gtatatgtta	tccattgggt	caatgtggac	780

62

cggcatgcat ctgttgagga agcataccaa gatggcaggt cccgactaaa catgttggtcta 840
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 cgcaagtttg gtacaccttt gaacaagtcg accatgacaa gtgatgagta taagaatgct 960
 gttctgcagg ctaaggaaca tattatggct ggggatatct tccagattgt ttttaagccag 1020
 5 aggttcgaga gacgaacata tgccaaccca tttgaggttt atcgagcatt acggattgtg 1080
 aatcctagcc catacaaggc gtatgtacag gcaagaggct gtgtattggt tgcgtctagt 1140
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 15 gcgcgcagcc acaacacgat gtactcatat aaagacgcag ataggcgctg ggaagggtgc 1680
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 gttagacaaag aatagttgtc tatggttata gtttagttct tgttcattgt tcttttcccc 1860
 actttccgtt aaaaaaagat gtcattagtg ggtggagaaa agcaataaga ctgttctcta 1920
 20 gaattcgagc tcggtaccgg atccaattcc cgatcgttca aacatttggc aataaagttt 1980
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 cgttaagcat gtaataatta acatgtaatg catgacgtta tttatgagat ggggttttat 2100
 gattagagtc ccgcaattat acatttaata cgcgatagaa aacaaaatat agcgcgcaaa 2160
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25

<210> 69

<211> 729

<212> PRT

<213> Artificial Sequence

30

<220>

<223> An A. tumefaciens mutant.

<400> 69

35 Met Val Thr Ile Ile Gln Asp Asp Gly Ala Glu Thr Tyr Glu Thr Lys

1

5

10

15

Gly Gly Ile Gln Val Ser Arg Lys Arg Arg Pro Thr Asp Tyr Ala Asn

20

25

30

Ala Ile Asp Asn Tyr Ile Glu Lys Leu Asp Ser His Arg Gly Ala Val

40

35

40

45

63

Phe	Lys	Ser	Asn	Tyr	Glu	Tyr	Pro	Gly	Arg	Tyr	Thr	Arg	Trp	Asp	Thr
50						55					60				
Ala	Ile	Val	Asp	Pro	Pro	Leu	Gly	Ile	Ser	Cys	Phe	Gly	Arg	Lys	Met
65					70				75					80	
5	Trp	Ile	Glu	Ala	Tyr	Asn	Gly	Arg	Gly	Glu	Val	Leu	Leu	Asp	Phe
					85				90					95	
Thr	Glu	Lys	Leu	Lys	Ala	Thr	Pro	Asp	Leu	Thr	Leu	Gly	Ala	Ser	Ser
			100					105					110		
Thr	Arg	Arg	Leu	Asp	Leu	Thr	Val	Asn	Glu	Pro	Asp	Arg	Val	Phe	Thr
10			115					120				125			
Glu	Glu	Glu	Arg	Ser	Lys	Ile	Pro	Thr	Val	Phe	Thr	Ala	Leu	Arg	Ala
			130			135					140				
Ile	Val	Asp	Leu	Phe	Tyr	Ser	Ser	Ala	Asp	Ser	Ala	Ile	Gly	Leu	Phe
145				150					155					160	
15	Gly	Ala	Phe	Gly	Tyr	Asp	Leu	Ala	Phe	Gln	Phe	Asp	Ala	Ile	Lys
					165				170					175	
Ser	Leu	Ala	Arg	Pro	Glu	Asp	Gln	Arg	Asp	Met	Val	Leu	Phe	Leu	Pro
			180					185					190		
Asp	Glu	Ile	Leu	Val	Val	Asp	His	Tyr	Ser	Ala	Lys	Ala	Trp	Ile	Asp
20			195					200				205			
Arg	Tyr	Asp	Phe	Glu	Lys	Asp	Gly	Met	Thr	Thr	Asp	Gly	Lys	Ser	Ser
			210			215					220				
Asp	Ile	Thr	Pro	Asp	Pro	Phe	Lys	Thr	Thr	Asp	Thr	Ile	Pro	Pro	Lys
225					230					235				240	
25	Gly	Asp	His	Arg	Pro	Gly	Glu	Tyr	Ser	Glu	Leu	Val	Val	Lys	Ala
				245						250				255	
Glu	Ser	Phe	Arg	Arg	Gly	Asp	Leu	Phe	Glu	Val	Val	Pro	Gly	Gln	Lys
			260				265						270		
Phe	Met	Glu	Arg	Cys	Glu	Ser	Asn	Pro	Ser	Ala	Ile	Ser	Arg	Arg	Leu
30			275				280					285			
Lys	Ala	Ile	Asn	Pro	Ser	Pro	Tyr	Ser	Phe	Phe	Ile	Asn	Leu	Gly	Asp
			290			295					300				
Gln	Glu	Tyr	Leu	Val	Gly	Ala	Ser	Pro	Glu	Met	Phe	Val	Arg	Val	Ser
305				310						315				320	
35	Gly	Arg	Arg	Ile	Glu	Thr	Cys	Pro	Ile	Ser	Gly	Thr	Ile	Lys	Arg
				325						330				335	
Asp	Asp	Pro	Ile	Ala	Asp	Ser	Glu	Gln	Ile	Leu	Lys	Leu	Leu	Asn	Ser
			340					345				350			
Lys	Lys	Asp	Glu	Ser	Glu	Leu	Thr	Met	Cys	Ser	Asp	Val	Asp	Arg	Asn
40			355					360					365		

64

Asp	Lys	Ser	Arg	Val	Cys	Glu	Pro	Gly	Ser	Val	Lys	Val	Ile	Gly	Arg	370	375	380	
Arg	Gln	Ile	Glu	Met	Tyr	Ser	Arg	Leu	Ile	His	Thr	Val	Asp	His	Ile	385	390	400	
5	Glu	Gly	Arg	Leu	Arg	Asp	Asp	Met	Asp	Ala	Phe	Asp	Gly	Phe	Leu	Ser	405	410	415
	His	Ala	Trp	Ala	Val	Thr	Val	Thr	Gly	Ala	Pro	Lys	Leu	Trp	Ala	Met	420	425	430
	Arg	Phe	Ile	Glu	Gly	His	Glu	Lys	Ser	Pro	Arg	Ala	Trp	Tyr	Gly	Gly	435	440	445
10	Ala	Ile	Gly	Met	Val	Gly	Phe	Asn	Gly	Asp	Met	Asn	Thr	Gly	Leu	Thr	450	455	460
	Leu	Arg	Thr	Ile	Arg	Ile	Lys	Asp	Gly	Ile	Ala	Glu	Val	Arg	Ala	Gly	465	470	480
15	Ala	Thr	Leu	Leu	Asn	Asp	Ser	Asn	Pro	Gln	Glu	Glu	Glu	Ala	Glu	Thr	485	490	495
	Glu	Leu	Lys	Ala	Ser	Ala	Met	Ile	Ser	Ala	Ile	Arg	Asp	Ala	Lys	Gly	500	505	510
	Thr	Asn	Ser	Ala	Ala	Thr	Lys	Arg	Asp	Ala	Ala	Lys	Val	Gly	Thr	Gly	515	520	525
20	Val	Lys	Ile	Leu	Leu	Val	Asp	His	Glu	Asp	Ser	Phe	Val	His	Thr	Leu	530	535	540
	Ala	Asn	Tyr	Phe	Arg	Gln	Thr	Gly	Ala	Thr	Val	Ser	Thr	Val	Arg	Ser	545	550	560
25	Pro	Val	Ala	Ala	Asp	Val	Phe	Asp	Arg	Phe	Gln	Pro	Asp	Leu	Val	Val	565	570	575
	Leu	Ser	Pro	Gly	Pro	Gly	Ser	Pro	Thr	Asp	Phe	Asp	Cys	Lys	Ala	Thr	580	585	590
	Ile	Lys	Ala	Ala	Arg	Ala	Arg	Asp	Leu	Pro	Ile	Phe	Gly	Val	Cys	Leu	595	600	605
30	Gly	Leu	Gln	Ala	Leu	Ala	Glu	Ala	Tyr	Gly	Gly	Glu	Leu	Arg	Gln	Leu	610	615	620
	Ala	Val	Pro	Met	His	Gly	Lys	Pro	Ser	Arg	Ile	Arg	Val	Leu	Glu	Pro	625	630	640
35	Gly	Leu	Val	Phe	Ser	Gly	Leu	Gly	Lys	Glu	Val	Thr	Val	Gly	Arg	Tyr	645	650	655
	His	Ser	Ile	Phe	Ala	Asp	Pro	Ala	Thr	Leu	Pro	Arg	Asp	Phe	Ile	Ile	660	665	670
	Thr	Ala	Glu	Ser	Glu	Asp	Gly	Thr	Ile	Met	Gly	Ile	Glu	His	Ala	Lys	675	680	685
40																			

65

Glu Pro Val Ala Ala Val Gln Phe His Pro Glu Ser Ile Met Thr Leu
 690 695 700
 Gly Gln Asp Ala Gly Met Arg Met Ile Glu Asn Val Val Val His Leu
 705 710 715 720
 5 Thr Arg Lys Ala Lys Thr Lys Ala Ala
 725

<210> 70

<211> 729

10 <212> PRT

<213> Artificial Sequence

<220>

<223> An A. tumefaciens mutant.

15

<400> 70

Met Val Thr Ile Ile Gln Asp Asp Gly Ala Glu Thr Tyr Glu Thr Lys
 1 5 10 15
 Gly Gly Ile Gln Val Ser Arg Lys Arg Arg Pro Thr Asp Tyr Ala Asn
 20 20 25 30
 Ala Ile Asp Asn Tyr Ile Glu Lys Leu Asp Ser His Arg Gly Ala Val
 35 40 45
 Phe Ser Ser Asn Tyr Glu Tyr Pro Gly Arg Tyr Thr Arg Trp Asp Thr
 50 55 60
 25 Ala Ile Val Asp Pro Pro Leu Gly Ile Ser Cys Phe Gly Arg Lys Met
 65 70 75 80
 Trp Ile Glu Ala Tyr Asn Gly Arg Gly Glu Val Leu Leu Asp Phe Ile
 85 90 95
 Thr Glu Lys Leu Lys Ala Thr Pro Asp Leu Thr Leu Gly Ala Ser Ser
 30 100 105 110
 Thr Arg Arg Leu Asp Leu Thr Val Asn Glu Pro Asp Arg Val Phe Thr
 115 120 125
 Glu Glu Glu Arg Ser Lys Ile Pro Thr Val Phe Thr Ala Leu Arg Ala
 130 135 140
 35 Ile Val Asp Leu Phe Tyr Ser Ser Ala Asp Ser Ala Ile Gly Leu Phe
 145 150 155 160
 Gly Ala Phe Gly Tyr Asp Leu Ala Phe Gln Phe Asp Ala Ile Lys Leu
 165 170 175
 Ser Leu Ala Arg Pro Glu Asp Gln Arg Asp Met Val Leu Phe Leu Pro
 40 180 185 190

66

Asp Glu Ile Leu Val Val Asp His Tyr Ser Ala Lys Ala Trp Ile Asp
 195 200 205
 Arg Tyr Asp Phe Glu Lys Asp Gly Met Thr Thr Asp Gly Lys Ser Ser
 210 215 220
 5 Asp Ile Thr Pro Asp Pro Phe Lys Thr Thr Asp Thr Ile Pro Pro Lys
 225 230 235 240
 Gly Asp His Arg Pro Gly Glu Tyr Ser Glu Leu Val Val Lys Ala Lys
 245 250 255
 Glu Ser Phe Arg Arg Gly Asp Leu Phe Glu Val Val Pro Gly Gln Lys
 10 260 265 270
 Phe Met Glu Arg Cys Glu Ser Asn Pro Ser Ala Ile Ser Arg Arg Leu
 275 280 285
 Lys Ala Ile Asn Pro Ser Pro Tyr Ser Ala Phe Ile Asn Leu Gly Asp
 290 295 300
 15 Gln Glu Tyr Leu Val Gly Ala Ser Pro Glu Met Phe Val Arg Val Ser
 305 310 315 320
 Gly Arg Arg Ile Glu Thr Cys Pro Ile Ser Gly Thr Ile Lys Arg Gly
 325 330 335
 Asp Asp Pro Ile Ala Asp Ser Glu Gln Ile Leu Lys Leu Leu Asn Ser
 20 340 345 350
 Lys Lys Asp Glu Ser Glu Leu Thr Met Cys Ser Asp Val Asp Arg Asn
 355 360 365
 Asp Lys Ser Arg Val Cys Glu Pro Gly Ser Val Lys Val Ile Gly Arg
 370 375 380
 25 Arg Gln Ile Glu Met Tyr Ser Arg Leu Ile His Thr Val Asp His Ile
 385 390 395 400
 Glu Gly Arg Leu Arg Asp Asp Met Asp Ala Phe Asp Gly Phe Leu Ser
 405 410 415
 His Ala Trp Ala Val Thr Val Thr Gly Ala Pro Lys Leu Trp Ala Met
 30 420 425 430
 Arg Phe Ile Glu Gly His Glu Lys Ser Pro Arg Ala Trp Tyr Gly Gly
 435 440 445
 Ala Ile Gly Met Val Gly Phe Asn Gly Asp Met Asn Thr Gly Leu Thr
 450 455 460
 35 Leu Arg Thr Ile Arg Ile Lys Asp Gly Ile Ala Glu Val Arg Ala Gly
 465 470 475 480
 Ala Thr Leu Leu Asn Asp Ser Asn Pro Gln Glu Glu Glu Ala Glu Thr
 485 490 495
 Glu Leu Lys Ala Ser Ala Met Ile Ser Ala Ile Arg Asp Ala Lys Gly
 40 500 505 510

67

Thr Asn Ser Ala Ala Thr Lys Arg Asp Ala Ala Lys Val Gly Thr Gly
 515 520 525
 Val Lys Ile Leu Leu Val Asp His Glu Asp Ser Phe Val His Thr Leu
 530 535 540
 5 Ala Asn Tyr Phe Arg Gln Thr Gly Ala Thr Val Ser Thr Val Arg Ser
 545 550 555 560
 Pro Val Ala Ala Asp Val Phe Asp Arg Phe Gln Pro Asp Leu Val Val
 565 570 575
 Leu Ser Pro Gly Pro Gly Ser Pro Thr Asp Phe Asp Cys Lys Ala Thr
 10 580 585 590
 Ile Lys Ala Ala Arg Ala Arg Asp Leu Pro Ile Phe Gly Val Cys Leu
 595 600 605
 Gly Leu Gln Ala Leu Ala Glu Ala Tyr Gly Gly Glu Leu Arg Gln Leu
 610 615 620
 15 Ala Val Pro Met His Gly Lys Pro Ser Arg Ile Arg Val Leu Glu Pro
 625 630 635 640
 Gly Leu Val Phe Ser Gly Leu Gly Lys Glu Val Thr Val Gly Arg Tyr
 645 650 655
 His Ser Ile Phe Ala Asp Pro Ala Thr Leu Pro Arg Asp Phe Ile Ile
 20 660 665 670
 Thr Ala Glu Ser Glu Asp Gly Thr Ile Met Gly Ile Glu His Ala Lys
 675 680 685
 Glu Pro Val Ala Ala Val Gln Phe His Pro Glu Ser Ile Met Thr Leu
 690 695 700
 25 Gly Gln Asp Ala Gly Met Arg Met Ile Glu Asn Val Val Val His Leu
 705 710 715 720
 Thr Arg Lys Ala Lys Thr Lys Ala Ala
 725

30 <210> 71

<211> 264

<212> DNA

<213> Artificial Sequence

35 <220>

<223> The sequence of a CTP.

<400> 71

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 40 gtcgctcctt tcaacggact taagtctctc gctgccttcc cagccaccgc caaggctaac 120

68

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aaccgacatta cttccatcac aagcaacggc ggaagagtta actgcatgca ggtgtggcct 180
cggattggaa agaagaagtt tgagactctc tcttaccttc ctgaccttac cgattccggc 240
ggtcgcgtca actgcatgca ggcc 264

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5 <210> 72

<211> 88

<212> PRT

<213> Artificial Sequence

10 <220>

<223> The sequence of a CTP.

<400> 72

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Gln Ala Thr Met Val Ala Pro Phe Asn Gly Leu Lys Ser Ser Ala Ala
          20          25          30
Phe Pro Ala Thr Arg Lys Ala Asn Asn Asp Ile Thr Ser Ile Thr Ser
          35          40          45
20 Asn Gly Gly Arg Val Asn Cys Met Gln Val Trp Pro Pro Ile Gly Lys
          50          55          60
Lys Lys Phe Glu Thr Leu Ser Tyr Leu Pro Asp Leu Thr Asp Ser Gly
65          70          75          80
Gly Arg Val Asn Cys Met Gln Ala
25          85

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<210> 73

<211> 264

<212> DNA

30 <213> Artificial Sequence

<220>

<223> The sequence of a CTP.

35 <400> 73

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gtcgtctcct tcaacggact taagtctctc gctgccttcc cagccaccgc caaggctaac 120
aaccgacatta cttccatcac aagcaacggc ggaagagtta actgcatgca ggtgtggcct 180
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40 ggtcgcgtca actgcatgca ggcc 264

```

<210> 74
 <211> 88
 <212> PRT
 5 <213> Artificial Sequence

<220>
 <223> The sequence of a CTP.

10 <400> 74
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 1 5 10 15
 Gln Ala Thr Met Val Ala Pro Phe Asn Gly Leu Lys Ser Ser Ala Ala
 20 25 30
 15 Phe Pro Ala Thr Arg Lys Ala Asn Asn Asp Ile Thr Ser Ile Thr Ser
 35 40 45
 Asn Gly Gly Arg Val Asn Cys Met Gln Val Trp Pro Pro Ile Glu Lys
 50 55 60
 Lys Lys Phe Glu Thr Leu Ser Tyr Leu Pro Asp Leu Thr Asp Ser Gly
 20 65 70 75 80
 Gly Arg Val Asn Cys Met Gln Ala
 85

<210> 75
 25 <211> 2190
 <212> DNA
 <213> Artificial Sequence

<220>
 30 <223> An optimized A. tumefaciens.

<400> 75
 atggtgacca tcattcagga tgacggtgcc gagacctacg agaccaaggg cggcatccag 60
 gtgagccgca agcgccgccc caccgattac gccaacgcc a tcgataacta catcgaaaag 120
 35 cttgatctcc atcgcggtgc cgtgttctcc tccaactacg aatccccagg ccgctacacc 180
 cgctgggata ccgcatctgt cgatccacca ctggcattt cctgcttcgg ccgcaagatg 240
 tggatcgaag cctacaacgg ccgcggcgaa gtgctgctcg atttcattac cgaaaagctg 300
 aaggccacac ccgatctcac cctcggcgct tcctccacc gccgcctcga tcttaccgtc 360
 aacgaaccag accgcgtctt caccgaagaa gaacgctcca aaatcccaac cgtcttcacc 420
 40 gctctcaggg ccacgtcgga cctcttctac tccagcgccg attccgcat ccgctcttc 480

70

ggtgccttcg gttacgatct cgccttccag ttcgacgcca tcaagcttct cctggcccgc	540
ccagaagacc agcgcgacat ggtgctgttc ctgcccgatg aaatcctcgt cgttgatcac	600
tactccgcc aaggcctggat cgaccgctac gatttcgaga aggacggcat gaccaccgac	660
ggcaaatctct ccgacattac ccccgatccc ttcagacca ccgataccat cccaccaag	720
5 ggcgatcacc gcccccgcga atactccgag ctgtgtgtga aggccaaagga aagcttcgcg	780
cgccggcacc tgttcgaggt cgttcccgcc cagaatctca tggagcgctg cgaaagcaac	840
ccatccgcc tttcccgccg cctgaaggcc atcaaccat cccctactc cttcttcac	900
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10 gccgacagcg agcagatttt gaaactgctc aactccaaaa aggacgaatc cgaactgacc	1080
atgtgctccg acgtggaccg caacgacaag agccgcgtct gcgagccagg ttccgtgaag	1140
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acggcctcta ccttcgcgac catccgcatc aaggacggta ttgccgaagt cgcgcgcggc	1440
gccaccctgc tcaacgatcc caaccacag gaagaagaag ccgaaccga actgaaggcc	1500
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ctgccaatct tcggcggttg cctcggtctg caggcattgg cagaagccta cggcgcgag	1860
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25 ggccctgctt tctccggtct cggcaaggaa gtcaccgtcg gtgcctacca ttccatcttc	1980
gccgatcccg ccacccctgcc acgcgatctc atcatcaccg cagaagcgga ggacggcacc	2040
atcatgggca tcgaacacgc caaggaaacca gtggccgcgg ttcagttcca cccagaatcc	2100
atcatgaccc tcgggtcagga cgcggcgatg cgcgatgatg agaacgtcgt ggtgcctcgt	2160
accgcgaagg ccaagaccaa ggccgcctga	2190

30

<210> 76

<211> 2160

<212> DNA

<213> *Rhodospseudomonas palustris*

35

<400> 76

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ctgctcgacc acccgccggg cgtgatgctg tcgctcgcca caaccgtgcc ggcccgctac	180
40 gagagcttcg acctcggtct tgcgatccg ccgctggcgc tcaccactag ggccgaaaaa	240

ttcaccatcg	aggcgctcaa	tccgcgcggc	cgggtgctga	tcgcgttctc	gtccgacaag	300
cttgaagagc	cctgcgtggt	ggtggagcag	gcctgcgcca	ccaagatcag	gggcccacatc	360
gtccgcggcg	aggccccggt	cgacgaagaa	caacgcaccc	gccgcgccag	cgcgatctcc	420
ctggtgcgcg	cggtagtgc	tgccttcgcc	tcgcccggcg	atccgatgct	cgggctgtac	480
5 ggcgccttcg	cctacgacct	tgtgttccag	ttcgaggatc	tgaagcagaa	gcgtgcccg	540
gaagccgacc	agcgcgacat	cgtgctgtac	gtgcccggatc	gcctgctggc	ctacgatcgc	600
gccaccggcc	gcggcgctga	catttcctac	gaattcgctc	ggaaggcgca	gtccacggcc	660
ggcctgccga	acgagaccgc	cgagagcgtc	tacaccaga	cggcgccgca	gggtttcgcc	720
gaccacgccc	cggcgcgacta	tcccaagggtg	gtcgagaagg	cccgcgcggc	gttcgcccg	780
10 ggcgacctgt	tcgaggcggt	gccgggcccag	ctgttcggcg	agccatgcga	cggttcggcg	840
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15 tgcaccgacg	tcgaccgcaa	cgacaaggcg	cgggtctgcg	tgcggggcac	gatcaaatgt	1140
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20 ggctccacca	tccgcaccat	ccgatgaag	gacggcctcg	ccgaagtctg	cgtcgccgc	1440
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gcactgttcc	aggcgctcg	cggcgatccc	gccaaagcgc	tgtcgcggtg	ggcgccggac	1560
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25 ctgaagatgc	tggccgaaaa	cagctatgat	cttctggtgc	tgtcgcccgg	tccggccgg	1740
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cgcggtctcc	cgaacgaggt	caccatcgcc	cgtaccact	cgtctatgt	cgacatgcgc	1980
30 gacatgcga	aggagctgac	cgtcacgcgc	tccaccgatg	acggcatcgc	gatggcgatc	2040
gagcacaaga	cctcgcgggt	cggcgccgtg	cagttccacc	ccgagtcgct	gatgtcgctc	2100
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<210> 77

35 <211> 773

<212> PRT

<213> Mesorhizobium loti

<400> 77

40 Met Glu Thr Ala Met Thr Met Lys Val Leu Glu Asn Gly Ala Glu Ser

72

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Phe Val Thr	Ala Gly Gly Ile Thr Ile	Thr Arg Glu Arg His Asp Arg	
	20	25	30
Pro Tyr Ala	Gly Ala Ile Asp Ala Tyr Val	Asp Gly Leu Asn Ser Arg	
5	35	40	45
Arg Gly Ala	Val Phe Ser Ser Asn Tyr Glu Tyr	Pro Gly Arg Tyr Thr	
	50	55	60
Arg Trp Asp	Thr Ala Ile Ile Asp Pro Pro	Leu Val Ile Ser Ala Arg	
65	70	75	80
10 Gly Arg	Ala Met Arg Ile Glu Ala Leu Asn	Arg Arg Gly Glu Ala Leu	
	85	90	95
Leu Pro Val	Ile Gly Lys Thr Leu Gly Gly Leu	Ala Asp Ile Thr Ile	
	100	105	110
Ala Glu Thr	Thr Lys Thr Leu Ile Arg Leu	Asp Val Ala Lys Pro Gly	
15	115	120	125
Arg Val Phe	Thr Glu Glu Glu Arg Ser Arg	Val Pro Ser Val Phe Thr	
	130	135	140
Val Leu Arg	Ala Ile Thr Ala Leu Phe Lys	Thr Asp Glu Asp Ala Asn	
145	150	155	160
20 Leu Gly	Leu Tyr Gly Ala Phe Gly Tyr	Asp Leu Ser Phe Gln Phe Asp	
	165	170	175
Pro Val Asp	Tyr Lys Leu Glu Arg Lys Pro	Ser Gln Arg Asp Leu Val	
	180	185	190
Leu Phe Leu	Pro Asp Glu Ile Leu Val Val	Asp His Tyr Ser Ala Lys	
25	195	200	205
Ala Trp Thr	Asp Arg Tyr Asp Tyr Ser Gly	Glu Gly Phe Ser Thr Glu	
	210	215	220
Gly Leu Pro	Arg Asp Ala Ile Ala Glu Pro	Phe Lys Thr Ala Asp Arg	
225	230	235	240
30 Ile Pro	Pro Arg Gly Asp His Glu Pro	Gly Glu Tyr Ala Asn Leu Val	
	245	250	255
Arg Arg Ala	Met Asp Ser Phe Lys Arg Gly	Asp Leu Phe Glu Val Val	
	260	265	270
Pro Gly Gln	Met Phe Tyr Glu Arg Cys Glu	Thr Gln Pro Ser Asp Ile	
35	275	280	285
Ser Arg Lys	Leu Lys Ser Ile Asn Pro	Ser Pro Tyr Ser Phe Phe Ile	
	290	295	300
Asn Leu Gly	Glu Asn Glu Tyr Leu Ile Gly	Ala Ser Pro Glu Met Phe	
305	310	315	320
40 Val Arg	Val Asn Gly Arg Arg Val Glu	Thr Cys Pro Ile Ser Gly Thr	

73

	325	330	335
Ile Lys Arg Gly Asp Asp Ala	Ile Ser Asp Ser Glu Gln Ile Leu Lys		
340	345	350	
Leu Leu Asn Ser Lys Lys Asp Glu Ser Glu Leu Thr Met Cys Ser Asp			
355	360	365	
5 Val Asp Arg Asn Asp Lys Ser Arg Val Cys Glu Pro Gly Ser Val Arg			
370	375	380	
Val Ile Gly Arg Arg Gln Ile Glu Met Tyr Ser Arg Leu Ile His Thr			
385	390	395	400
10 Val Asp His Ile Glu Gly Arg Leu Arg Glu Gly Met Asp Ala Phe Asp			
405	410	415	
Ala Phe Leu Ser His Ala Trp Ala Val Thr Val Thr Gly Ala Pro Lys			
420	425	430	
Leu Trp Ala Met Arg Phe Ile Glu Gln Asn Glu Lys Ser Pro Arg Ala			
435	440	445	
15 Trp Tyr Gly Gly Ala Ile Gly Met Val Asn Phe Asn Gly Asp Met Asn			
450	455	460	
Thr Gly Leu Thr Leu Arg Thr Ile Arg Ile Lys Asp Gly Ile Ala Glu			
465	470	475	480
20 Val Arg Ala Gly Ala Thr Leu Leu Phe Asp Ser Ile Pro Glu Glu Glu			
485	490	495	
Glu Ala Glu Thr Glu Leu Lys Ala Ser Ala Met Leu Ser Ala Ile Arg			
500	505	510	
Asp Ala Lys Thr Gly Asn Ser Ala Ser Thr Glu Arg Thr Thr Ala Arg			
515	520	525	
25 Val Gly Asp Gly Val Asn Ile Leu Leu Val Asp His Glu Asp Ser Phe			
530	535	540	
Val His Thr Leu Ala Asn Tyr Phe Arg Gln Thr Gly Ala Asn Val Ser			
545	550	555	560
30 Thr Val Arg Thr Pro Val Pro Asp Glu Val Phe Glu Arg Leu Lys Pro			
565	570	575	
Asp Leu Val Val Leu Ser Pro Gly Pro Gly Thr Pro Lys Asp Phe Asp			
580	585	590	
Cys Ala Ala Thr Ile Arg Arg Ala Arg Ala Arg Asp Leu Pro Ile Phe			
595	600	605	
35 Gly Val Cys Leu Gly Leu Gln Ala Leu Ala Glu Ala Tyr Gly Gly Glu			
610	615	620	
Leu Arg Gln Leu His Ile Pro Met His Gly Lys Pro Ser Arg Ile Arg			
625	630	635	640
40 Val Ser Lys Pro Gly Ile Ile Phe Ser Gly Leu Pro Lys Glu Val Thr			

[illegible]

<210> 78

<211> 732

15 <212> PRT

<213> Azospirillum brasilense

<400> 78

	Met	Tyr	Pro	Ala	Asp	Leu	Leu	Ala	Ser	Pro	Asp	Leu	Leu	Glu	Pro	Leu
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	Arg	Phe	Gln	Thr	Arg	Gly	Gly	Val	Thr	Val	Thr	Arg	Arg	Ala	Thr	Ala
				20					25						30	
	Leu	Asp	Pro	Arg	Thr	Ala	Leu	Asp	Pro	Val	Ile	Asp	Ala	Leu	Asp	Arg
				35				40						45		
25	Arg	Arg	Gly	Leu	Leu	Leu	Ser	Ser	Gly	Val	Glu	Ala	Pro	Gly	Arg	Tyr
				50				55					60			
	Arg	Arg	His	Ala	Leu	Gly	Phe	Thr	Asp	Pro	Ala	Val	Ala	Leu	Thr	Ala
						70						75				80
	Arg	Gly	Arg	Thr	Leu	Arg	Ile	Asp	Ala	Leu	Asn	Gly	Arg	Gly	Gln	Val
30					85					90					95	
	Leu	Leu	Pro	Ala	Val	Ala	Glu	Ala	Leu	Arg	Gly	Leu	Glu	Ala	Leu	Ala
					100				105					110		
	Gly	Leu	Glu	Glu	Ala	Pro	Ser	Arg	Val	Thr	Ala	Ser	Ser	Ala	Ser	Pro
					115				120				125			
35	Ala	Pro	Leu	Pro	Gly	Glu	Glu	Arg	Ser	Arg	Gln	Pro	Ser	Val	Phe	Ser
					130			135					140			
	Val	Leu	Arg	Ala	Val	Leu	Asp	Leu	Phe	Ala	Ala	Pro	Asp	Asp	Pro	Leu
						150					155				160	
	Leu	Gly	Leu	Tyr	Gly	Ala	Phe	Ala	Tyr	Asp	Leu	Ala	Phe	Gln	Phe	Glu
40					165					170					175	

75

Pro Ile Arg Gln Arg Leu Glu Arg Pro Asp Asp Gln Arg Asp Leu Leu
 180 185 190
 Leu Tyr Ieu Pro Asp Arg Leu Val Ala Leu Asp Pro Ile Ala Gly Leu
 195 200 205
 5 Ala Arg Leu Val Ala Tyr Glu Phe Ile Thr Ala Ala Gly Ser Thr Glu
 210 215 220
 Gly Leu Glu Cys Gly Gly Arg Asp His Pro Tyr Arg Pro Asp Thr Asn
 225 230 235 240
 Ala Glu Ala Gly Cys Asp His Ala Pro Gly Asp Tyr Gln Arg Val Val
 10 245 250 255
 Glu Ser Ala Lys Ala Ala Phe Arg Arg Gly Asp Leu Phe Glu Val Val
 260 265 270
 Pro Gly Gln Thr Phe Ala Glu Pro Cys Ala Asp Ala Pro Ser Ser Val
 275 280 285
 15 Phe Arg Arg Leu Arg Ala Ala Asn Pro Ala Pro Tyr Glu Ala Phe Val
 290 295 300
 Asn Leu Gly Arg Gly Glu Phe Leu Val Ala Ala Ser Pro Glu Met Tyr
 305 310 315 320
 Val Arg Val Ala Gly Gly Arg Val Glu Thr Cys Pro Ile Ser Gly Thr
 20 325 330 335
 Val Ala Arg Gly Ala Asp Ala Leu Gly Asp Ala Ala Gln Val Leu Arg
 340 345 350
 Leu Leu Thr Ser Ala Lys Asp Ala Ala Glu Leu Thr Met Cys Thr Asp
 355 360 365
 25 Val Asp Arg Asn Asp Lys Ala Arg Val Cys Glu Pro Gly Ser Val Arg
 370 375 380
 Val Ile Gly Arg Arg Met Ile Glu Leu Tyr Ser Arg Leu Ile His Thr
 385 390 395 400
 Val Asp His Val Glu Gly Arg Leu Arg Ser Gly Met Asp Ala Leu Asp
 30 405 410 415
 Ala Phe Leu Thr His Ser Trp Ala Val Thr Val Thr Gly Ala Pro Lys
 420 425 430
 Arg Trp Ala Met Gln Phe Leu Glu Asp Thr Glu Gln Ser Pro Arg Arg
 435 440 445
 35 Trp Tyr Gly Gly Ala Phe Gly Arg Leu Gly Phe Asp Gly Gly Met Asp
 450 455 460
 Thr Gly Leu Thr Leu Arg Thr Ile Arg Met Ala Glu Gly Val Ala Tyr
 465 470 475 480
 Val Arg Ala Gly Ala Thr Leu Leu Ser Asp Ser Asp Pro Asp Ala Glu
 40 485 490 495

76

Asp Ala Glu Cys Arg Leu Lys Ala Ala Ala Phe Arg Asp Ala Ile Arg
 500 505 510
 Gly Thr Ala Ala Gly Ala Ala Pro Thr Leu Pro Ala Ala Pro Arg Gly
 515 520 525
 5 Gly Glu Gly Arg Arg Val Leu Leu Val Asp His Asp Asp Ser Phe Val
 530 535 540
 His Thr Leu Ala Asp Tyr Leu Arg Gln Thr Gly Ala Ser Val Thr Thr
 545 550 555 560
 Leu Arg His Ser His Ala Arg Ala Ala Leu Ala Glu Arg Arg Pro Asp
 10 565 570 575
 Leu Val Val Leu Ser Pro Gly Pro Gly Arg Pro Ala Asp Phe Asp Val
 580 585 590
 Ala Gly Thr Ile Asp Ala Ala Leu Ala Leu Gly Leu Pro Val Phe Gly
 595 600 605
 15 Val Cys Leu Gly Leu Gln Gly Met Val Glu Arg Phe Gly Gly Ala Leu
 610 615 620
 Asp Val Leu Pro Glu Pro Val His Gly Lys Ala Thr Glu Val Arg Val
 625 630 635 640
 Leu Gly Gly Ala Leu Phe Ala Gly Leu Pro Glu Arg Leu Thr Val Gly
 20 645 650 655
 Arg Tyr His Ser Leu Val Ala Arg Asp Arg Leu Pro Ala Asp Leu
 660 665 670
 Thr Val Thr Ala Glu Thr Ala Asp Gly Leu Val Met Ala Val Glu His
 675 680 685
 25 Arg Arg Leu Pro Leu Ala Ala Val Gln Phe His Pro Glu Ser Ile Leu
 690 695 700
 Ser Leu Asp Gly Gly Ala Gly Leu Ala Leu Leu Gly Asn Val Met Asp
 705 710 715 720
 Arg Leu Ala Ala Gly Ala Leu Thr Asp Ala Ala Ala
 30 725 730

<210> 79

<211> 731

<212> PRT

35 <213> *Brucella melitensis*

<400> 79

Met Asn Ala Lys Thr Ala Asp Ser Glu Ile Phe Gln His Glu Thr Ala
 1 5 10 15
 40 Gly Gly Ile Ile Val Glu Arg Val Arg His Leu Thr Ala Tyr Lys Gly

77

	20		25		30
	Ala Ile Glu Ser Tyr Ile Asp Val Leu Asn Glu Trp Arg Gly Ala Val				
	35		40		45
	Phe Ser Ser Asn Tyr Glu Tyr Pro Gly Arg Tyr Thr Arg Trp Asp Thr				
5	50		55		60
	Ala Ile Val Asp Pro Pro Val Val Ile Thr Ser Arg Ala Arg Thr Met				
65		70		75	80
	Arg Ile Glu Ala Leu Asn Ala Arg Gly Val Ile Leu Leu Arg Pro Ile				
	85		90		95
10	Leu Asp Thr Val Lys Ala Leu Ser Glu Val Lys Ile Asp Gln Ser Gly				
	100		105		110
	Glu Asn Arg Ile Asp Leu Thr Ile Val Glu Pro Val Gly Thr Phe Thr				
	115		120		125
	Glu Glu Glu Arg Ser Arg Met Pro Ser Val Phe Thr Val Leu Arg Ala				
15	130		135		140
	Ile Val Gly Leu Phe Phe Ser Glu Glu Asp Ala Asn Leu Gly Leu Tyr				
145		150		155	160
	Gly Ala Phe Gly Tyr Asp Leu Ala Phe Gln Phe Asp Pro Ile Gln Tyr				
	165		170		175
20	Lys Leu Lys Arg Pro Asp Asp Gln Arg Asp Leu Val Leu Phe Ile Pro				
	180		185		190
	Asp Glu Ile Phe Val Ala Asp His Tyr Ala Ala Arg Ala Trp Val Asp				
	195		200		205
	Arg Tyr Glu Phe Arg Cys Gly Gly Ser Ser Thr His Gly Leu Asp Arg				
25	210		215		220
	Ala Thr Pro Val Val Pro Phe Lys Pro Ser Glu Arg Lys Leu Ala Arg				
225		230		235	240
	Gly Asp His Asn Pro Gly Glu Tyr Ala Arg Leu Val Glu Arg Ala Lys				
	245		250		255
30	Glu Ser Phe Lys Arg Gly Asp Leu Phe Glu Val Val Pro Gly Gln Thr				
	260		265		270
	Phe Tyr Glu Arg Cys His Thr Ala Pro Ser Glu Ile Phe Arg Arg Leu				
	275		280		285
	Lys Ser Ile Asn Pro Ser Pro Tyr Ser Phe Phe Ile Asn Leu Gly Glu				
35	290		295		300
	Ser Glu Tyr Leu Val Gly Ala Ser Pro Glu Met Phe Val Arg Val Asn				
305		310		315	320
	Gly Arg Arg Ile Glu Thr Cys Pro Ile Ser Gly Thr Ile Lys Arg Gly				
	325		330		335
40	Glu Asp Ala Ile Ser Asp Ser Glu Gln Ile Leu Lys Leu Leu Asn Ser				

	340		345		350
	Lys Lys Asp Glu Ser Glu Leu Thr Met Cys Ser Asp Val Asp Arg Asn				
	355		360		365
	Asp Lys Ser Arg Val Cys Glu Pro Gly Ser Val Arg Val Ile Gly Arg				
5	370		375		380
	Arg Gln Ile Glu Met Tyr Ser Arg Leu Ile His Thr Val Asp His Ile				
	385		390		395
	Glu Gly Arg Leu Arg Asp Gly Met Asp Ala Phe Asp Gly Phe Leu Ser				
	405		410		415
10	His Ala Trp Ala Val Thr Val Thr Gly Ala Pro Lys Leu Trp Ala Met				
	420		425		430
	Arg Phe Leu Glu Glu Asn Glu Arg Ser Pro Arg Ala Trp Tyr Gly Gly				
	435		440		445
	Ala Ile Gly Met Met His Phe Asn Gly Asp Met Asn Thr Gly Leu Thr				
15	450		455		460
	Leu Arg Thr Ile Arg Ile Lys Asp Gly Val Ala Glu Ile Arg Ala Gly				
	465		470		475
	Ala Thr Leu Leu Phe Asp Ser Asn Pro Asp Glu Glu Glu Ala Glu Thr				
	485		490		495
20	Glu Leu Lys Ala Ser Ala Met Ile Ala Ala Val Arg Asp Ala Gln Lys				
	500		505		510
	Ser Asn Gln Ile Ala Glu Glu Ser Val Ala Ala Lys Val Gly Glu Gly				
	515		520		525
	Val Ser Ile Leu Leu Val Asp His Glu Asp Ser Phe Val His Thr Leu				
25	530		535		540
	Ala Asn Tyr Phe Arg Gln Thr Gly Ala Lys Val Ser Thr Val Arg Ser				
	545		550		555
	Pro Val Ala Glu Glu Ile Phe Asp Arg Val Asn Pro Asp Leu Val Val				
	565		570		575
30	Leu Ser Pro Gly Pro Gly Ser Pro Gln Asp Phe Asp Cys Lys Ala Thr				
	580		585		590
	Ile Asp Lys Ala Arg Lys Arg Gln Leu Pro Ile Phe Gly Val Cys Leu				
	595		600		605
	Gly Leu Gln Ala Leu Ala Glu Ala Tyr Gly Gly Ala Leu Arg Gln Leu				
35	610		615		620
	Arg Val Pro Val His Gly Lys Pro Ser Arg Ile Arg Val Ser Lys Pro				
	625		630		635
	Glu Arg Ile Phe Ser Gly Leu Pro Glu Glu Val Thr Val Gly Arg Tyr				
	645		650		655
40	His Ser Ile Phe Ala Asp Pro Glu Arg Leu Pro Asp Asp Phe Leu Val				

79

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        660                665                670
Thr Ala Glu Thr Glu Asp Gly Ile Ile Met Ala Phe Glu His Lys His
        675                680                685
Glu Pro Val Ala Ala Val Gln Phe His Pro Glu Ser Ile Met Thr Leu
5   690                695                700
Gly His Asn Ala Gly Met Arg Met Ile Glu Asn Ile Val Thr His Leu
705                710                715                720
Ala Gly Lys His Lys Ala Arg Arg Thr Asn Tyr
        725                730

10
<210> 80
<211> 735
<212> PRT
<213> Nostoc sp.

15
<400> 80
Met Ile Ala Asp Ser His Ser Tyr Arg Thr Asn Gly Asn Val Arg Val
 1           5           10           15
Ser Arg Ser Ile Thr Gln Val Lys Met Glu Thr Ala Leu Glu Glu Ile
20           20           25           30
Leu Phe Tyr Leu Asn Ser Gln Arg Gly Gly Leu Leu Thr Ser Ser Tyr
        35           40           45
Glu Tyr Pro Gly Arg Tyr Lys Arg Trp Ala Ile Gly Phe Val Asn Pro
        50           55           60
25 Pro Val Glu Leu Ser Thr Ser Gly Asn Thr Phe Thr Leu Thr Ala Leu
        65           70           75           80
Asn Glu Arg Gly Tyr Val Leu Leu Pro Val Ile Phe Glu Cys Leu Ser
        85           90           95
Lys Ser Glu Gln Leu Gln Lys Leu Thr Glu His His His Lys Ile Thr
30           100          105          110
Gly Leu Val Lys Ser Thr Pro Glu Phe Phe Ala Glu Glu Glu Arg Ser
        115          120          125
Lys Gln Pro Ser Thr Phe Thr Val Ile Arg Glu Ile Leu His Ile Phe
        130          135          140
35 Ser Ser Gln Glu Asp Glu His Leu Gly Leu Tyr Gly Ala Phe Gly Tyr
        145          150          155          160
Asp Leu Val Phe Gln Phe Glu Gln Ile Thr Gln Cys Leu Glu Arg Pro
        165          170          175
Gln Asp Gln Arg Asp Leu Val Leu Tyr Leu Pro Asp Glu Leu Ile Val
40           180           185           190

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80

Val Asp Tyr Tyr Gln Gln Gln Ala Phe Arg Leu Glu Tyr Asp Phe Ile
 195 200 205
 Thr Ala His Gly Ser Thr Tyr Asp Leu Pro Arg Thr Gly Glu Ser Val
 210 215 220
 5 Asp Tyr Arg Gly Gln Cys Leu Thr Pro Pro Gln Asn Ala Asp His Lys
 225 230 235 240
 Ile Gly Glu Tyr Ala Lys Leu Val Glu Phe Ala Leu Asp Tyr Phe Arg
 245 250 255
 Arg Gly Asp Leu Phe Glu Val Val Pro Ser Gln Asn Phe Phe Thr Ala
 10 260 265 270
 Cys Glu Ala Pro Pro Ser Gln Leu Phe Glu Thr Leu Lys Gln Ile Asn
 275 280 285
 Pro Ser Pro Tyr Gly Phe Ile Phe Asn Leu Gly Gly Glu Tyr Ile Ile
 290 295 300
 15 Gly Ala Ser Pro Glu Met Phe Val Arg Val Glu Gly Arg Arg Val Glu
 305 310 315 320
 Thr Cys Pro Ile Ser Gly Thr Ile Thr Arg Gly His Asp Ala Ile Asp
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 Asp Ala Val Gln Ile Arg Gln Leu Leu Asn Ser His Lys Asp Glu Ala
 20 340 345 350
 Glu Leu Thr Met Cys Thr Asp Val Asp Arg Asn Asp Lys Ser Arg Ile
 355 360 365
 Cys Glu Pro Gly Ser Val Lys Val Ile Gly Arg Arg Gln Ile Glu Leu
 370 375 380
 25 Tyr Ser His Leu Ile His Thr Val Asp His Val Glu Gly Ile Leu Arg
 385 390 395 400
 Pro Glu Phe Asp Ala Leu Asp Ala Phe Leu Ser His Thr Trp Ala Val
 405 410 415
 Thr Val Thr Gly Ala Pro Lys Arg Ala Ala Ile Gln Phe Ile Glu Lys
 30 420 425 430
 Asn Glu Arg Ser Val Arg Arg Trp Tyr Gly Gly Ala Val Gly Tyr Leu
 435 440 445
 Asn Phe Asn Gly Asn Leu Asn Thr Gly Leu Ile Leu Arg Thr Ile Arg
 450 455 460
 35 Leu Gln Asp Ser Ile Ala Glu Val Arg Val Gly Ala Thr Leu Leu Tyr
 465 470 475 480
 Asp Ser Ile Pro Gln Ala Glu Glu Gln Glu Thr Ile Thr Lys Ala Ala
 485 490 495
 Ala Ala Phe Glu Thr Ile Arg Arg Ala Lys Gln Ile Asp Pro Gln Ile
 40 500 505 510

81

Glu Glu Ser Ser Thr Arg Lys Leu Ser Lys Tyr Leu Pro Asp Gly Gln
 515 520 525
 Ser Gly Lys His Ile Leu Leu Ile Asp His Glu Asp Ser Phe Val His
 530 535 540
 5 Thr Leu Ala Asn Tyr Ile Arg Ser Thr Gly Ala Thr Val Thr Thr Leu
 545 550 555 560
 Arg His Gly Phe Ser Glu Ser Leu Phe Asp Thr Glu Arg Pro Asp Leu
 565 570 575
 Val Val Leu Ser Pro Gly Pro Gly Arg Pro Ser Glu Phe Lys Val Gln
 10 580 585 590
 Glu Thr Val Ala Ala Cys Val Arg Arg Gln Ile Pro Leu Phe Gly Val
 595 600 605
 Cys Leu Gly Leu Gln Gly Ile Val Glu Ala Phe Gly Gly Glu Leu Gly
 610 615 620
 15 Val Leu Asn Tyr Pro Gln His Gly Lys Ser Ser Arg Ile Phe Val Thr
 625 630 635 640
 Ala Pro Asp Ser Val Met Phe Gln Asp Leu Pro Glu Ser Phe Thr Val
 645 650 655
 Gly Arg Tyr His Ser Leu Phe Ala Leu Ser Gln Arg Leu Pro Lys Glu
 20 660 665 670
 Leu Lys Val Thr Ala Ile Ser Asp Asp Glu Val Ile Met Ala Ile Glu
 675 680 685
 His Gln Thr Leu Pro Ile Ala Ala Val Gln Phe His Pro Glu Ser Ile
 690 695 700
 25 Met Thr Leu Ala Gly Glu Val Gly Leu Met Met Ile Lys Asn Val Val
 705 710 715 720
 Gln Lys Tyr Thr Gln Ser Gln Gln Ser Thr Val Pro Ile Tyr Asp
 725 730 735

30 <210> 81

<211> 715

<212> PRT

<213> Nostoc sp.

35 <400> 81

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 Asp Glu Ile Leu Phe His Leu Asn Gln Val Arg Gly Gly Leu Leu Thr
 20 25 30
 40 Ser Ser Tyr Glu Tyr Pro Gly Arg Tyr Lys Arg Trp Ala Ile Gly Phe

	35		40		45	
	Ile Asn Pro Pro Leu Gln Leu Thr Thr Arg Glu Asn Ala Phe Thr Ile					
	50		55		60	
	Ser Ser Leu Asn Pro Arg Gly Gln Val Leu Leu Pro Thr Leu Phe Gln					
5	65		70		75	80
	His Leu Ser Ala Gln Ser Gln Leu Gln Gln Ile Ser Leu Asn His Asp					
		85		90		95
	Tyr Ile Thr Gly Glu Ile Arg Pro Thr Lys Gln Leu Phe Thr Glu Glu					
		100		105		110
10	Gln Arg Ser Lys Gln Pro Ser Ala Phe Thr Val Ile Arg Glu Ile Leu					
		115		120		125
	Gln Ile Phe Ala Ser Asp Glu Asp Glu His Leu Gly Leu Tyr Gly Ala					
		130		135		140
	Phe Gly Tyr Asp Leu Val Phe Gln Phe Glu Pro Ile Pro Gln Lys Ile					
15	145		150		155	160
	Ala Arg Pro Ala Asp Gln Arg Asp Leu Val Leu Tyr Leu Pro Asp Glu					
		165		170		175
	Leu Ile Val Val Asp Tyr Tyr Leu Gln Lys Ala Tyr Arg His Gln Tyr					
		180		185		190
20	Glu Phe Ala Thr Glu His Gly Asn Thr Glu His Leu Pro Arg Thr Gly					
		195		200		205
	Gln Ser Ile Asp Tyr Gln Gly Lys His Leu Leu Pro Asn Gln Thr Ala					
		210		215		220
	Asp His Gln Pro Gly Glu Tyr Ala Asn Leu Val Glu Gln Ala Leu Asp					
25	225		230		235	240
	Tyr Phe Arg Arg Gly Asp Leu Phe Glu Val Val Pro Ser Gln Asn Phe					
		245		250		255
	Phe Thr Ala Cys Glu Gln Ser Pro Ser Gln Leu Phe Gln Thr Leu Arg					
		260		265		270
30	Gln Ile Asn Pro Ser Pro Tyr Gly Phe Leu Leu Asn Leu Gly Gly Glu					
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	Tyr Leu Ile Gly Ala Ser Pro Glu Met Phe Val Arg Val Asp Gly Arg					
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	Arg Val Glu Thr Cys Pro Ile Ser Gly Thr Ile Arg Arg Gly Glu Asp					
35	305		310		315	320
	Ala Leu Gly Asp Ala Val Gln Ile Arg Gln Leu Leu Asn Ser His Lys					
		325		330		335
	Asp Glu Ala Glu Leu Thr Met Cys Thr Asp Val Asp Arg Asn Asp Lys					
		340		345		350
40	Ser Arg Ile Cys Glu Pro Gly Ser Val Arg Val Ile Gly Arg Arg Gln					

	355		360		365
	Ile Glu Leu Tyr Ser His Leu Ile His Thr Val Asp His Val Glu Gly				
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	Ile Leu Arg Pro Glu Phe Asp Ala Leu Asp Ala Phe Leu Ser His Thr				
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	Trp Ala Val Thr Val Thr Gly Ala Pro Lys Arg Ala Ala Met Gln Phe				
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10	Gly Tyr Leu Gly Phe Asn Gly Asn Leu Asn Thr Gly Leu Thr Leu Arg				
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	Thr Ile Arg Leu Gln Asp Ser Ile Ala Glu Val Arg Val Gly Ala Thr				
	450		455		460
	Val Leu Tyr Asp Ser Ile Pro Ser Ala Glu Glu Glu Glu Thr Ile Thr				
15	465		470		475
	Lys Ala Thr Ala Leu Phe Glu Thr Ile Arg Arg His Thr Thr Ala Asn				
		485		490	495
	Lys Thr Gln Gly Asn Asp Ser His Arg Pro Gly Asp Ile Ala His Asn				
	500		505		510
20	Lys Arg Ile Leu Leu Ile Asp Tyr Glu Asp Ser Phe Val His Thr Leu				
	515		520		525
	Ala Asn Tyr Ile Arg Thr Thr Gly Ala Thr Val Thr Thr Leu Arg His				
	530		535		540
	Gly Phe Ala Glu Ser Tyr Phe Asp Ala Glu Arg Pro Asp Leu Val Val				
25	545		550		555
	Leu Ser Pro Gly Pro Gly Arg Pro Ser Asp Phe Arg Val Pro Gln Thr				
		565		570	575
	Val Ala Ala Leu Val Gly Arg Glu Ile Pro Ile Phe Gly Val Cys Leu				
	580		585		590
30	Gly Leu Gln Gly Ile Val Glu Ala Phe Gly Gly Glu Leu Gly Val Leu				
	595		600		605
	Asp Tyr Pro Gln His Gly Lys Pro Ala Arg Ile Ser Val Thr Ala Pro				
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	Asp Ser Val Leu Phe Gln Asn Leu Pro Ala Ser Phe Ile Val Gly Arg				
35	625		630		635
	Tyr His Ser Leu Phe Ala Gln Pro Gln Thr Ile Pro Gly Glu Leu Lys				
		645		650	655
	Val Thr Ala Ile Ser Glu Asp Asn Val Ile Met Ala Ile Glu His Gln				
	660		665		670
40	Thr Leu Pro Ile Ala Ala Val Gln Phe His Pro Glu Ser Ile Met Thr				

84

675 680 685
 Leu Ala Gly Glu Val Gly Gln Thr Ile Ile Lys Asn Val Val Gln Thr
 690 695 700
 Tyr Thr Gln Thr Leu Glu Thr Ser Ile Tyr Ser
 5 705 710 715

<210> 82

<211> 719

<212> PRT

10 <213> *Rhodopseudomonas palustris*

<400> 82

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 20 25 30
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 35 40 45
 Met Leu Ser Ser Gly Thr Thr Val Pro Gly Arg Tyr Glu Ser Phe Asp
 20 50 55 60
 Leu Gly Phe Ala Asp Pro Pro Leu Ala Leu Thr Thr Arg Ala Glu Lys
 65 70 75 80
 Phe Thr Ile Glu Ala Leu Asn Pro Arg Gly Arg Val Leu Ile Ala Phe
 85 90 95
 25 Leu Ser Asp Lys Leu Glu Glu Pro Cys Val Val Val Glu Gln Ala Cys
 100 105 110
 Ala Thr Lys Ile Arg Gly His Ile Val Arg Gly Glu Ala Pro Val Asp
 115 120 125
 Glu Glu Gln Arg Thr Arg Arg Ala Ser Ala Ile Ser Leu Val Arg Ala
 30 130 135 140
 Val Ile Ala Ala Phe Ala Ser Pro Ala Asp Pro Met Leu Gly Leu Tyr
 145 150 155 160
 Gly Ala Phe Ala Tyr Asp Leu Val Phe Gln Phe Glu Asp Leu Lys Gln
 165 170 175
 35 Lys Arg Ala Arg Glu Ala Asp Gln Arg Asp Ile Val Leu Tyr Val Pro
 180 185 190
 Asp Arg Leu Leu Ala Tyr Asp Arg Ala Thr Gly Arg Gly Val Asp Ile
 195 200 205
 Ser Tyr Glu Phe Ala Trp Lys Gly Gln Ser Thr Ala Gly Leu Pro Asn
 40 210 215 220

85

	Glu Thr Ala Glu Ser Val Tyr Thr Gln Thr Gly Arg Gln Gly Phe Ala	
225	230	240
	Asp His Ala Pro Gly Asp Tyr Pro Lys Val Val Glu Lys Ala Arg Ala	
	245	255
5	Ala Phe Ala Arg Gly Asp Leu Phe Glu Ala Val Pro Gly Gln Leu Phe	
	260	270
	Gly Glu Pro Cys Glu Arg Ser Pro Ala Glu Val Phe Lys Arg Leu Cys	
	275	285
	Arg Ile Asn Pro Ser Pro Tyr Gly Gly Leu Leu Asn Leu Gly Asp Gly	
10	290	300
	Glu Phe Leu Val Ser Ala Ser Pro Glu Met Phe Val Arg Ser Asp Gly	
	305	320
	Arg Arg Ile Glu Thr Cys Pro Ile Ser Gly Thr Ile Ala Arg Gly Val	
	325	335
15	Asp Ala Ile Ser Asp Ala Glu Gln Ile Gln Lys Leu Leu Asn Ser Glu	
	340	350
	Lys Asp Glu Phe Glu Leu Asn Met Cys Thr Asp Val Asp Arg Asn Asp	
	355	365
	Lys Ala Arg Val Cys Val Pro Gly Thr Ile Lys Val Leu Ala Arg Arg	
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	Gln Ile Glu Thr Tyr Ser Lys Leu Phe His Thr Val Asp His Val Glu	
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	Gly Met Leu Arg Pro Gly Phe Asp Ala Leu Asp Ala Phe Leu Thr His	
	405	415
25	Ala Trp Ala Val Thr Val Thr Gly Ala Pro Lys Leu Trp Ala Met Gln	
	420	430
	Phe Val Glu Asp His Glu Arg Ser Pro Arg Arg Trp Tyr Ala Gly Ala	
	435	445
	Phe Gly Val Val Gly Phe Asp Gly Ser Ile Asn Thr Gly Leu Thr Ile	
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	Arg Thr Ile Arg Met Lys Asp Gly Leu Ala Glu Val Arg Val Gly Ala	
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	Thr Cys Leu Phe Asp Ser Asn Pro Val Ala Glu Asp Lys Glu Cys Gln	
	485	495
35	Val Lys Ala Ala Ala Leu Phe Gln Ala Leu Arg Gly Asp Pro Ala Lys	
	500	510
	Pro Leu Ser Ala Val Ala Pro Asp Ala Thr Gly Ser Gly Lys Lys Val	
	515	525
	Leu Leu Val Asp His Asp Asp Ser Phe Val His Met Leu Ala Asp Tyr	
40	530	540

86

Phe Arg Gln Val Gly Ala Gln Val Thr Val Val Arg Tyr Val His Gly
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 Leu Lys Met Leu Ala Glu Asn Ser Tyr Asp Leu Leu Val Leu Ser Pro
 565 570 575
 5 Gly Pro Gly Arg Pro Glu Asp Phe Lys Ile Lys Asp Thr Ile Asp Ala
 580 585 590
 Ala Leu Ala Lys Lys Leu Pro Ile Phe Gly Val Cys Leu Gly Val Gln
 595 600 605
 Ala Met Gly Glu Tyr Phe Gly Gly Thr Leu Gly Gln Leu Ala Gln Pro
 10 610 615 620
 Ala His Gly Arg Pro Ser Arg Ile Gln Val Arg Gly Gly Ala Leu Met
 625 630 635 640
 Arg Gly Leu Pro Asn Glu Val Thr Ile Gly Arg Tyr His Ser Leu Tyr
 645 650 655
 15 Val Asp Met Arg Asp Met Pro Lys Glu Leu Thr Val Thr Ala Ser Thr
 660 665 670
 Asp Asp Gly Ile Ala Met Ala Ile Glu His Lys Thr Leu Pro Val Gly
 675 680 685
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 705 710 715

<210> 83

25 <211> 2160

<212> DNA

<213> Rhodospseudomonas palustris

<400> 83

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	ggcgacctgt	tcgaggcggt	gccggggccag	ctgttcggcg	agccatgcga	gcggctcgccg	840
	gccgaagtgt	tcaagcgggt	gtgccggatc	aaccgcgtcg	cctatggcgg	cctgtccaat	900
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	cgccggatcg	agacctgccc	gatctcggc	actatcgccc	gcggcgctga	tgcgatcagc	1020
	gatgctgagc	agatccagaa	gctcttgaa	tcgagaagg	acgagttcga	gctgaatatg	1080
	tgacccgacg	tcgaccgcaa	cgacaaggcg	cgggtctgcg	tgccgggcac	gatcaaatgt	1140
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<210> 84

<211> 2190

<212> DNA

30 <213> Artificial Sequence

<220>

<223> An A. tumefaciens mutant.

35 <400> 84

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	cttgattccc	atcgccggcg	gtttttttcg	tccaaactat	aatatccggg	ccgttacacc	180
	cgtcgggata	cggccatcgt	cgatccggcg	ctcggcattt	ctgtttttgg	ccgcaagatg	240
40	tgatcgaa	ccataatgg	cccgccgcaa	gtgctgctcg	atcttcattac	ggaaaaagctg	300

88

	aaggcgacac	cgatctcac	cctcgcgct	tctcgaccc	gccgctcga	tcttaccgtc	360
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	gctctcagag	ccatcgtcga	cctcttctat	tcgagcgagg	atcgcccat	cggcctgttc	480
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<210> 85

35 <211> 2190

<212> DNA

<213> Artificial Sequence

<220>

40 <223> An A. tumefaciens mutant.

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<210> 86

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5

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<400> 86

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91

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<210> 89

<211> 2190

<212> DNA

25 <213> Artificial Sequence

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<223> An A. tumefaciens mutant.

30 <400> 89

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30 <211> 2190

<212> DNA

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35 <223> An A. tumefaciens mutant.

<400> 90

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35

<210> 91

<211> 2190

<212> DNA

<213> Artificial Sequence

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<220>

<223> An *A. tumefaciens* mutant.

<400> 91

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98

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10 <210> 93

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15 <220>

<223> An A. tumefaciens mutant.

<400> 93

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<212> DNA

20 <213> *Oryza sativa*

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15 <210> 95

<211> 1498

<212> DNA

<213> *Oryza sativa*

20 <400> 95

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<212> DNA

10 <213> Zea mays

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10 <211> 504

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<400> 97

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20 tttttagtgt tcattcatctt tcccccagtt cattttggaa agttgttcat cgttttttca 360
ccgagttcat attggggaaa aaaagcaata ccgttttctg gtcctttgaa atgaataaat 420
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aaaaaaaaaa aaaaaaaaaa aata 504

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25 <210> 98

<211> 2161

<212> DNA

<213> *Nicotiana tabacum*

30 <400> 98

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35 ttatggatga ggacaggttc attgaagctt ctaaaagcgg gaacttgatt ccgctgcaca 300
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gtgttggtgc ctacagcgtg gtgggggctc aaccatctat ggaaattgtg gctaaggaaac 480
acaatgtgac tatattgtac caccacactg gaaaattgac ccagaagact gtccaagatc 540
40 ccatgacgat tccgaggagt atttctgagg gatggaagcc cagactcatt gatgaacttc 600

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103

ctgataccctt ttgtggtgga tgggttggtt atttctcata tgacacagtt cggtagttag 660
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 aattaggact atacgaagat gtcatttgtt ttgatcatgt tgagaagaaa gcacatgtga 780
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 gttctgtgga tttctgtact catgcttttg gaccttcatt aaccaaggga aacatgacaa 960
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 a 2161

<210> 99

30 <211> 606

<212> PRT

<213> Oryza sativa

<400> 99

35 Met Glu Ser Ile Ala Ala Ala Thr Phe Thr Pro Ser Arg Leu Ala Ala
 1 5 10 15
 Arg Pro Ala Thr Pro Ala Ala Ala Ala Pro Val Arg Ala Arg Ala
 20 25 30
 Ala Val Ala Ala Gly Gly Arg Arg Arg Thr Ser Arg Arg Gly Gly Val
 40 35 40 45

104

Arg	Cys	Ser	Ala	Gly	Lys	Pro	Glu	Ala	Ser	Ala	Val	Ile	Asn	Gly	Ser
50						55					60				
Ala	Ala	Ala	Arg	Ala	Ala	Glu	Glu	Asp	Arg	Arg	Arg	Phe	Phe	Glu	Ala
65					70					75				80	
5	Ala	Glu	Arg	Gly	Ser	Gly	Lys	Gly	Asn	Leu	Val	Pro	Met	Trp	Glu
				85						90				95	
Ile	Val	Ser	Asp	His	Leu	Thr	Pro	Val	Leu	Ala	Tyr	Arg	Cys	Leu	Val
				100					105				110		
Pro	Glu	Asp	Asn	Met	Glu	Thr	Pro	Ser	Phe	Leu	Phe	Glu	Ser	Val	Glu
10				115				120				125			
Gln	Gly	Pro	Glu	Gly	Thr	Thr	Asn	Val	Gly	Arg	Tyr	Ser	Met	Val	Gly
				130			135				140				
Ala	His	Pro	Val	Met	Glu	Val	Val	Ala	Lys	Glu	His	Lys	Val	Thr	Ile
145					150					155				160	
15	Met	Asp	His	Glu	Lys	Gly	Lys	Val	Thr	Glu	Gln	Val	Val	Asp	Asp
					165					170				175	
Met	Gln	Ile	Pro	Arg	Ser	Met	Met	Glu	Gly	Trp	His	Pro	Gln	Gln	Ile
					180				185				190		
Asp	Gln	Leu	Pro	Asp	Ser	Phe	Thr	Gly	Gly	Trp	Val	Gly	Phe	Phe	Ser
20			195					200				205			
Tyr	Asp	Thr	Val	Arg	Tyr	Val	Glu	Lys	Lys	Lys	Leu	Pro	Phe	Ser	Gly
						210		215			220				
Ala	Pro	Gln	Asp	Asp	Arg	Asn	Leu	Pro	Asp	Val	His	Leu	Gly	Leu	Tyr
225					230					235				240	
25	Asp	Asp	Val	Leu	Val	Phe	Asp	Asn	Val	Glu	Lys	Lys	Val	Tyr	Val
					245					250				255	
His	Trp	Val	Asn	Leu	Asp	Arg	His	Ala	Thr	Thr	Glu	Asp	Ala	Phe	Gln
					260				265				270		
Asp	Gly	Lys	Ser	Arg	Leu	Asn	Leu	Leu	Leu	Ser	Lys	Val	His	Asn	Ser
30				275				280				285			
Asn	Val	Pro	Lys	Leu	Ser	Pro	Gly	Phe	Val	Lys	Leu	His	Thr	Arg	Gln
						290		295			300				
Phe	Gly	Thr	Pro	Leu	Asn	Lys	Ser	Thr	Met	Thr	Ser	Asp	Glu	Tyr	Lys
305					310					315				320	
35	Asn	Ala	Val	Met	Gln	Ala	Lys	Glu	His	Ile	Met	Ala	Gly	Asp	Ile
					325					330				335	
Gln	Ile	Val	Leu	Ser	Gln	Arg	Phe	Glu	Arg	Gln	Thr	Tyr	Ala	Asn	Pro
					340				345				350		
Phe	Glu	Val	Tyr	Arg	Ala	Leu	Arg	Ile	Val	Asn	Pro	Ser	Pro	Tyr	Met
40				355				360				365			

105

Ala Tyr Val Gln Ala Arg Gly Cys Val Leu Val Ala Ser Ser Pro Glu
 370 375 380

Ile Leu Thr Arg Val Arg Lys Gly Lys Ile Ile Asn Arg Pro Leu Ala
 385 390 395 400

5 Gly Thr Val Arg Arg Gly Lys Thr Glu Lys Glu Asp Glu Met Gln Glu
 405 410 415

Gln Gln Leu Leu Ser Asp Glu Lys Gln Cys Ala Glu His Ile Met Leu
 420 425 430

Val Asp Leu Gly Arg Asn Asp Val Gly Lys Val Ser Lys Pro Gly Ser
 10 435 440 445

Val Lys Val Glu Lys Leu Met Asn Ile Glu Arg Tyr Ser His Val Met
 450 455 460

His Ile Ser Ser Thr Val Ser Gly Glu Leu Asp Asp His Leu Gln Ser
 465 470 475 480

15 Trp Asp Ala Leu Arg Ala Ala Leu Pro Val Gly Thr Val Ser Gly Ala
 485 490 495

Pro Lys Val Lys Ala Met Glu Leu Ile Asp Glu Leu Glu Val Thr Arg
 500 505 510

Arg Gly Pro Tyr Ser Gly Gly Leu Gly Gly Ile Ser Phe Asp Gly Asp
 20 515 520 525

Met Leu Ile Ala Leu Ala Leu Arg Thr Ile Val Phe Ser Thr Ala Pro
 530 535 540

Ser His Asn Thr Met Tyr Ser Tyr Lys Asp Thr Glu Arg Arg Arg Glu
 545 550 555 560

25 Trp Val Ala His Leu Gln Ala Gly Ala Gly Ile Val Ala Asp Ser Ser
 565 570 575

Pro Asp Asp Glu Gln Arg Glu Cys Glu Asn Lys Ala Ala Ala Leu Ala
 580 585 590

Arg Ala Ile Asp Leu Ala Glu Ser Ala Phe Val Asp Lys Glu
 30 595 600 605

<210> 100

<211> 67

<212> PRT

35 <213> Oryza sativa

<400> 100

Met Cys Val Leu Val Ala Ala Ala Val Arg Glu Glu Glu Ser Lys Phe
 1 5 10 15

40 Lys Ala Gly Ala Ala Glu Gly Cys Asn Ile Leu Pro Leu Lys Arg Cys

106

20 25 30
 Ile Phe Ser Asp His Leu Thr Pro Val Leu Ala Tyr Arg Cys Leu Val
 35 40 45
 Arg Glu Asp Asp Arg Glu Ala Pro Ser Phe Leu Phe Glu Ser Val Glu
 5 50 55 60
 Gln Gly Ser
 65

 <210> 101
 10 <211> 525
 <212> PRT
 <213> Zea mays

 <400> 101
 15 Met Trp Glu Cys Ile Lys Gly Asn Leu Val Pro Met Trp Glu Cys Ile
 1 5 10 15
 Val Ser Asp His Leu Thr Pro Val Leu Ala Tyr Arg Cys Leu Val Pro
 20 25 30
 Glu Asp Asn Val Asp Ala Pro Ser Phe Leu Phe Glu Ser Val Glu Gln
 20 35 40 45
 Gly Pro Gln Gly Thr Thr Asn Val Gly Arg Tyr Ser Met Val Gly Ala
 50 55 60
 His Pro Val Met Glu Ile Val Ala Lys Asp His Lys Val Thr Ile Met
 65 70 75 80
 25 Asp His Glu Lys Ser Gln Val Thr Glu Gln Val Val Asp Asp Pro Met
 85 90 95
 Gln Ile Pro Arg Thr Met Met Glu Gly Trp His Pro Gln Gln Ile Asp
 100 105 110
 Glu Leu Pro Glu Ser Phe Ser Gly Gly Trp Val Gly Phe Phe Ser Tyr
 30 115 120 125
 Asp Thr Val Arg Tyr Val Glu Lys Lys Lys Leu Pro Phe Ser Ser Ala
 130 135 140
 Pro Gln Asp Asp Arg Asn Leu Pro Asp Val His Leu Gly Leu Tyr Asp
 145 150 155 160
 35 Asp Val Leu Val Phe Asp Asn Val Glu Lys Lys Val Tyr Val Ile His
 165 170 175
 Trp Val Asn Val Asp Arg His Ala Ser Val Glu Glu Ala Tyr Gln Asp
 180 185 190
 Gly Arg Ser Arg Leu Asn Met Leu Leu Ser Lys Val His Asn Ser Asn
 40 195 200 205

107

Val Pro Thr Leu Ser Pro Gly Phe Val Lys Leu His Thr Arg Lys Phe
 210 215 220
 Gly Thr Pro Leu Asn Lys Ser Thr Met Thr Ser Asp Glu Tyr Lys Asn
 225 230 235 240
 5 Ala Val Leu Gln Ala Lys Glu His Ile Met Ala Gly Asp Ile Phe Gln
 245 250 255
 Ile Val Leu Ser Gln Arg Phe Glu Arg Arg Thr Tyr Ala Asn Pro Phe
 260 265 270
 Glu Val Tyr Arg Ala Leu Arg Ile Val Asn Pro Ser Pro Tyr Met Ala
 10 275 280 285
 Tyr Val Gln Ala Arg Gly Cys Val Leu Val Ala Ser Ser Pro Glu Ile
 290 295 300
 Leu Thr Arg Val Ser Lys Gly Lys Ile Ile Asn Arg Pro Leu Ala Gly
 305 310 315 320
 15 Thr Val Arg Arg Gly Lys Thr Glu Lys Glu Asp Gln Met Gln Glu Gln
 325 330 335
 Gln Leu Leu Ser Asp Glu Lys Gln Cys Ala Glu His Ile Met Leu Val
 340 345 350
 Asp Leu Gly Arg Asn Asp Val Gly Lys Val Ser Lys Pro Gly Gly Ser
 20 355 360 365
 Val Lys Val Glu Lys Leu Ile Ile Glu Arg Tyr Ser His Val Met His
 370 375 380
 Ile Ser Ser Thr Val Ser Gly Gln Leu Asp Asp His Leu Gln Ser Trp
 385 390 395 400
 25 Asp Ala Leu Arg Ala Ala Leu Pro Val Gly Thr Val Ser Gly Ala Pro
 405 410 415
 Lys Val Lys Ala Met Glu Leu Ile Asp Lys Leu Glu Val Thr Arg Arg
 420 425 430
 Gly Pro Tyr Ser Gly Gly Leu Gly Gly Ile Ser Phe Asp Gly Asp Met
 30 435 440 445
 Gln Ile Ala Leu Ser Leu Arg Thr Ile Val Phe Ser Thr Ala Pro Ser
 450 455 460
 His Asn Thr Met Tyr Ser Tyr Lys Asp Ala Asp Arg Arg Arg Glu Trp
 465 470 475 480
 35 Val Ala His Leu Gln Ala Gly Ala Gly Ile Val Ala Asp Ser Ser Pro
 485 490 495
 Asp Asp Glu Gln Arg Glu Cys Glu Asn Lys Ala Ala Leu Ala Arg
 500 505 510
 Ala Ile Asp Leu Ala Glu Ser Ala Phe Val Asn Lys Glu
 40 515 520 525

108

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<210> 102
<211> 92
<212> PRT
5 <213> Triticum aestivum

<400> 102
Pro Asn Ser Gly Gly Leu Gly Gly Ile Ser Phe Asp Gly Asp Met Leu
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10 Ile Ala Leu Ala Leu Arg Thr Ile Val Phe Ser Thr Ala Pro Ser Pro
    20             25             30
    Asn Arg Met Tyr Ser Tyr Lys Ser Ser Asp Arg Pro Arg Glu Trp Val
        35             40             45
    Ala His Leu Gln Ala Gly Ala Gly Ile Val Ala Asp Ser Ile Pro Asp
15    50             55             60
    Asp Glu Gln Lys Glu Phe Glu Asn Lys Ala Ala Ala Leu Ala Arg Ala
    65             70             75             80
    Ile Asp Leu Ala Glu Ser Ala Phe Leu Asp Lys Glu
        85             90

20
<210> 103
<211> 616
<212> PRT
<213> Nicotiana tabacum

25
<400> 103
Met Gln Ser Leu Pro Ile Ser Tyr Arg Leu Phe Pro Ala Thr His Arg
  1             5             10             15
    Lys Val Leu Pro Phe Ala Val Ile Ser Ser Arg Ser Ser Thr Ser Ala
30    20             25             30
    Leu Ala Leu Arg Val Arg Thr Leu Gln Cys Arg Cys Leu His Ser Ser
        35             40             45
    Ser Leu Val Met Asp Glu Asp Arg Phe Ile Glu Ala Ser Lys Ser Gly
        50             55             60
35 Asn Leu Ile Pro Leu His Lys Thr Ile Phe Ser Asp His Leu Thr Pro
    65             70             75             80
    Val Leu Ala Tyr Arg Cys Leu Val Lys Glu Asp Asp Arg Glu Ala Pro
        85             90             95
    Ser Phe Leu Phe Glu Ser Val Glu Pro Gly Phe Arg Gly Ser Ser Val
40    100             105             110

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109

Gly Arg Tyr Ser Val Val Gly Ala Gln Pro Ser Met Glu Ile Val Ala
 115 120 125
 Lys Glu His Asn Val Thr Ile Leu Asp His His Thr Gly Lys Leu Thr
 130 135 140
 5 Gln Lys Thr Val Gln Asp Pro Met Thr Ile Pro Arg Ser Ile Ser Glu
 145 150 155 160
 Gly Trp Lys Pro Arg Leu Ile Asp Glu Leu Pro Asp Thr Phe Cys Gly
 165 170 175
 Gly Trp Val Gly Tyr Phe Ser Tyr Asp Thr Val Arg Tyr Val Glu Asn
 10 180 185 190
 Arg Lys Leu Pro Phe Leu Arg Ala Pro Glu Asp Asp Arg Asn Leu Ala
 195 200 205
 Asp Ile Gln Leu Gly Leu Tyr Glu Asp Val Ile Val Phe Asp His Val
 210 215 220
 15 Glu Lys Lys Ala His Val Ile His Trp Val Gln Leu Asp Gln Tyr Ser
 225 230 235 240
 Ser Leu Pro Glu Ala Tyr Leu Asp Gly Lys Lys Arg Leu Glu Ile Leu
 245 250 255
 Val Ser Arg Val Gln Gly Ile Glu Ser Pro Arg Leu Ser Pro Gly Ser
 20 260 265 270
 Val Asp Phe Cys Thr His Ala Phe Gly Pro Ser Leu Thr Lys Gly Asn
 275 280 285
 Met Thr Ser Glu Glu Tyr Lys Asn Ala Val Leu Gln Ala Lys Glu His
 290 295 300
 25 Ile Ala Ala Gly Asp Ile Phe Gln Ile Val Leu Ser Gln Arg Phe Glu
 305 310 315 320
 Arg Arg Thr Phe Ala Asp Pro Phe Glu Val Tyr Arg Ala Leu Arg Ile
 325 330 335
 Val Asn Pro Ser Pro Tyr Met Thr Tyr Ile Gln Ala Arg Gly Cys Ile
 30 340 345 350
 Leu Val Ala Ser Ser Pro Glu Ile Leu Thr Arg Val Lys Lys Arg Arg
 355 360 365
 Ile Val Asn Arg Pro Leu Ala Gly Thr Ser Arg Gly Lys Thr Pro
 370 375 380
 35 Asp Glu Asp Val Met Leu Glu Met Gln Met Leu Lys Asp Glu Lys Gln
 385 390 395 400
 Arg Ala Glu His Ile Met Leu Val Asp Leu Gly Arg Asn Asp Val Gly
 405 410 415
 Lys Val Ser Lys Pro Gly Ser Val Asn Val Glu Lys Leu Met Ser Val
 40 420 425 430

110

Glu Arg Tyr Ser His Val Met His Ile Ser Ser Thr Val Ser Gly Glu			
435	440	445	
Leu Leu Asp His Leu Thr Cys Trp Asp Ala Leu Arg Ala Ala Leu Pro			
450	455	460	
5 Val Gly Thr Val Ser Gly Ala Pro Lys Val Lys Ala Met Glu Leu Ile			
465	470	475	480
Asp Gln Leu Glu Val Ala Arg Arg Gly Pro Tyr Ser Gly Gly Phe Gly			
485	490	495	
Gly Ile Ser Phe Ser Gly Asp Met Asp Ile Ala Leu Ala Leu Arg Thr			
10 500	505	510	
Met Val Phe Leu Asn Gly Ala Arg Tyr Asp Thr Met Tyr Ser Tyr Thr			
515	520	525	
Asp Ala Ser Lys Arg Gln Glu Trp Val Ala His Leu Gln Ser Gly Ala			
530	535	540	
15 Gly Ile Val Ala Asp Ser Asn Pro Asp Glu Glu Gln Ile Glu Cys Glu			
545	550	555	560
Asn Lys Val Ala Gly Leu Cys Arg Ala Ile Asp Leu Ala Glu Ser Ala			
565	570	575	
Phe Val Lys Gly Arg His Lys Pro Ser Val Lys Ile Asn Gly Ser Val			
20 580	585	590	
Pro Asn Leu Phe Ser Arg Val Gln Arg Gln Thr Ser Val Met Ser Lys			
595	600	605	
Asp Arg Val His Glu Lys Arg Asn			
610	615		